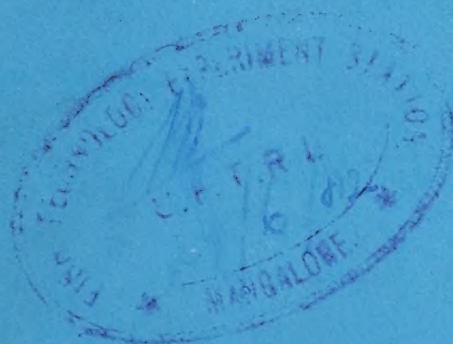


ISSN: 0015—3001

Fishery Technology

JULY, 1981

VOLUME 18, No. 2



Society of Fisheries Technologists (India)

28/6/82

Fishery Technology

July 1981

Volume 18, No. 2

ACKNOWLEDGEMENT

Consequent on the election of Shri N. Unnikrishnan Nair, Scientist, Central Institute of Fisheries Technology, Cochin as Editor, *Fishery Technology* in April 1981, Shri S. Gopalan Nayar, Scientist has relinquished the office of Editor, *Fishery Technology* with effect from April 1981.

Shri S. Gopalan Nayar was Editor from June 1972 to April 1981. During this long span of 10 years Shri Nayar has rendered very valuable services to the Journal as Editor. The Society of Fisheries Technologists (India) and the Editorial Board of *Fishery Technology* record their sincere thanks to Shri S. Gopalan Nayar for the services rendered by him to *Fishery Technology* as Editor.



Cochin: Society of Fisheries Technologists (India)

Society of Fisheries Technologists (India)

Cochin-682 029

OFFICERS AND COMMITTEE, 1981

President

C. C. Panduranga Rao

Vice Presidents

M. R. Nair

N. G. Magar

Secretary

R. Balasubramanyan

Assistant Secretary

A. Vasanth Shenoy

Treasurer

G. R. Unnithan

Executive Committee

P. Appukutta Panicker

K. K. Balachandran

T. S. Gopalakrishna Iyer

K. Gopakumar

M. K. Kandoran

K. Radhalakshmi

K. Ravindran

M. Shahul Hammeed

P. Vasudeva Prabhu

Society of Fisheries Technologists (India) is established to promote the cause of fisheries in general and fisheries technology in particular in all its aspects by (a) providing a forum for fishery technologists connected with production and processing of fish, design and construction of fishing boats and gear and manufacture of engines, auxiliaries and deck equipments, to meet periodically for discussion, exchange of information, findings and experience on different aspects of fisheries technology, (b) encouraging original investigations on problems related to fisheries technology, (c) publishing a journal called "Fishery Technology", (d)

instituting prizes in recognition of outstanding contributions to the advancement of fisheries technology in India and (e) such other means consistent with the aims of the society as the executive committee may decide.

Membership shall be open to all persons interested in fisheries, fisheries technology and fishing industry. Details of further facilities accorded to members and forms of application for membership are available from the Secretary, Society of Fisheries Technologists (India), Matsyapuri P. O., Cochin-682 029, INDIA.

Fishery Technology is published and distributed by the Society of Fisheries Technologists (India). It is published twice a year in January and July.

Single copy of each issue of the journal will be supplied free to the bonafide members of the society. For others, the rate of subscription for the journal is as follows:

Overseas	US \$ 12
Inland	Rs. 40

Terms are cash with order or against proforma invoice. Orders and subscriptions should be sent to "Society of Fisheries Technologists (India)."

Claims regarding issues lost or damaged in transit should be addressed to the Editor,

Fishery Technology, Matsyapuri P. O., Cochin-682 029, India. Claims cannot be entertained if they are received later than three months after the date of posting plus such time as would be expected for transit by post.

Enquiries for back volumes of the journal should be addressed to the Editor, Fishery Technology, Matsyapuri P. O., Cochin-682 029, India.

The Indian Council of Agricultural Research, New Delhi has given a generous grant of Rs. 7,500/- for 1980-1981 towards the publication of Fishery Technology.

The society and the editorial board assume no responsibility for the statements and views expressed by contributors.

Contents of Volume 18, No. 2, 1981

	PAGE		PAGE
Oxidation of residual lipids in fish protein concentrates and its effect on the nutritional quality of the protein		Studies on blue discolouration in canned body meat of crab (<i>Scylla serrata</i>)	
K. Devadasan	71	P. K. Vijayan & K. K. Balachandran	117
Biology of fouling in Neendakara Port, a tropical estuary in the south west coast of India		Influence of sex, spawning, starvation and water temperature on fatty acid composition in <i>Tilapia mossambica</i>	
K. Dharmaraj & N. Balakrishnan Nair	79	K. G. Ramachandran Nair & K. Gopakumar	123
The influence of plating technique and incubation temperature on the bacterial count from fish and fishery products		Comparative study of the nutrient content of fish and shell fish	
Nirmala Thampuran, H. Krishna Iyer & K. Mahadeva Iyer	95	M. K. Mukundan, A. G. Radhakrishnan, M. A. James & M. R. Nair	129
Seasonal changes in the biochemical composition of ovary in <i>Heteropneustes fossilis</i> (Bloch)		Selection of bacterial flora in the chlor-tetracycline treated oil sardine (<i>Sardinella longiceps</i>), Indian mackerel (<i>Rastrelliger kanagurta</i>) and prawn (<i>Metapenaeus dobsoni</i>) during ice storage	
Kalpana D. Shreni	101	P. K. Surendran & K. Gopakumar	133
Microbiological evaluation and keeping quality of fishes reared in livestock sewage fed ponds without artificial feed		On the vertical distribution of seers and other commercially important fishes in the surface drift nets	
V. N. Bachhil	105	Percy Dawson, J. Sitarama Rao & Y. Sreekrishna	143
Survey on the idle capacity of fish processing (freezing) plants in India		Book review	
I. West coast			
H. Krishna Iyer, P. Srinivasa Rao, G. R. Unnithan, A. K. Kesavan Nair & R. G. Nair	109		145

INDEX TO AUTHORS

Bachhil, V. N.	105	Nair, M. R.	129
Balachandran, K. K.	117	Nair, R. G.	109
Balakrishnan Nair, N.	79, 145	Nirmala Thampuran	95
Devadasan, K.	71	Percy Dawson	143
Dharmaraj, K.	79	Radhakrishnan, A. G.	129
Gopakumar, K.	123, 133	Ramachandran Nair, K. G.	123
James, M. A.	129	Sitarama Rao, J.	143
Kalpana D. Shreni	101	Sreekrishna, Y.	143
Kesavan Nair, A. K.	109	Srinivasa Rao, P.	109
Krishna Iyer, H.	95, 109	Surendran, P. K.	133
Mahadeva Iyer, K.	95	Unnithan, G. R.	109
Mukundan, M. K.	129	Vijayan, P. K.	117

The following papers appeared in the last issue of Fishery Technology
Contents of Volume 18, No. 1, 1981

	PAGE		PAGE
Spiny lobsters and their fishing techniques		Studies on the chemical control of psychrophilic bacterial spoilage of fish. ii- The effect of antibiotics on the growth of psychrophilic bacteria isolated from marine fish	
K. V. Mohan Rajan, B. Meenakumari & R. Balasubramanyan	1	C. P. Anand & T. M. Rudra Setty	41
Studies on Raschel knotless netting		Studies on the chemical control of psychrophilic bacterial spoilage of fish. iii- The effect of chemical preservatives on the growth of psychrophilic bacteria isolated from marine fish	
S. Gopalan Nayar & K. Radhalakshmi	13	C. P. Anand & T. M. Rudra Setty	47
Estimation of fish production from Hirakud reservoir		Studies on the chemical control of psychrophilic bacterial spoilage of fish. iv- The effect of chemical preservatives on the growth of psychrophilic bacteria isolated from marine fish	
M. D. Varghese, A. K. Kesavan Nair, V. C. George & A. A. Khan	17	C. P. Anand & T. M. Rudra Setty	55
Observations on the lunar and tidal influence on gill netting in the Bay of Bengal		Prediction of drained weight in canned prawn under commercial conditions	
S. Pati	25	D. R. Chaudhuri, S. K. Bhattacharya & A. N. Bose	59
Comparative study of traditional and improved containers for transportation of fresh fish		On the importance of <i>Mesopodopsis zeylanica</i> (Crustacea, Mysidacea) as food of fish	
C. C. Panduranga Rao, D. Imam Khasim Saheb, S. S. Gupta, G. R. Unnithan, S. T. Chari, R. Srinivasan, T. Santhanaraj & K. V. N. Pillai	29	M. D. Varghese	61
Effect of frozen storage on the physical properties of corrugated fibre-board master cartons and waxed duplex cartons		Isolation of <i>Salmonella larochelle</i> for the first time in India	
T. K. Srinivasa Gopal & T. K. Govindan	35	T. S. Gopalakrishna Iyer & S. P. Damle	63

INSTRUCTIONS TO AUTHORS

Papers submitted for publication in *Fishery Technology* should be typewritten, double space on one side of the paper only, in duplicate. The manuscript should be in the final form and no alterations or additions are allowed at the proof stage. Typescripts that are not concise or do not conform to the conventions of *Fishery Technology* will be returned to the author for revision. Papers should be sent to the Editor, Fishery Technology, Matsyapuri P. O., Cochin-682 029, India.

Contributions from authors who are members of the Society of Fisheries Technologists (India) and who do not owe dues of any sort to the Society alone will be considered for publication. Non-members who are desirous of publishing papers in *Fishery Technology* shall enroll themselves as members of the Society paying the prescribed subscription and admission fees.

Original research papers, short notes and review articles on fishery technology or any other branch of marine science will be accepted for publication.

Papers should be headed by a concise but informative full title, by the names of the authors (name in full with initials for each author) and the name and address of the establishment where the work was performed (foot notes, indicating present addresses may be included on the title page). Titles of papers should be sufficiently descriptive and informative about the contents so that the titles can be used in indexing and in coding for information, storage and retrieval. Authors should also give a shortened version of the titles not exceeding 60 letters and spaces suitable for a running title.

Papers are divided into the following sections:

(a) The *synopsis* should contain a brief but informative summary of the contents and conclusions of the paper and

should also highlight any new information contained in the paper. Normally the synopsis should not exceed 250 words and should be in the form acceptable to abstracting journals; (b) *Introduction* should contain the purpose of the investigation and its relation to other works in the same field; (c) *Materials and Methods*; (d) *Results and Discussion*. The results may be presented in the text or in tables or in figures and should be described with a minimum of discussion. Both tables and figures illustrating the same results will not be permitted. Drawings or illustrations should be made in Indian ink on tracing paper, approximately twice the dimension of the finished product as it will appear in the journal (usually single column width); (e) *Acknowledgements*; (f) *References*. The Harvard system, not the numbering system should be used for the citation of references in the text, as follows: For paper written by one or two authors, as 'Finch (1956)' or '(Finch, 1956)' or 'Brewer & Stewer (1942)' or '(Brewer & Stewer, 1942)'. For papers written by three or more authors as, 'Smith *et al.* (1957)' or '(Smith *et al.*, 1957)'. Where more than one paper by the same authors, it should be given as 'Brewer & Stewer (1956 a, b)' or '(Brewer & Stewer, 1956 a, b)'. At the end of the paper, references should be listed in *alphabetical* order except for papers by three or more authors (which are given in the text only as *et al.*), which should be grouped in chronological order after any other papers by the first author. The authors' initials should be included, but not the title of the paper. Examples:

Finch, G. I. (1956) *J. Sci. Ind. Res.* **15**, 539

Pope, C. G. & Stevens, M. F. (1939)
Biochem. J. **33**, 1070

Kerbs, H. A., Speake, R. N. & Hems, R.
(1965) *Biochem. J.* **94**, 712

Mavinkurve, S. S., Gangal, S. V., Savant,
P. L. & Kumta, U. S. (1967) *J. Fd
Sci.* **32**, 711

It should be noted that the year, volume number and first page should be cited for references.

Titles of journals should be abbreviated in accordance with World List of Scientific Periodicals, 4th edition and supplements.

References to books and monographs should include names of editors, edition number, volume number, relevant page numbers, name of publisher and town where published. Examples:

Benyami, M. (1959) in *Modern Fishing Gear of the World* (Kristjonsson, H., Ed.), Vol. 1, p. 213, Fishing News (Books) Ltd., London

Schultz, H. W. (1960) *Food Enzymes*, 1st edn., p. 65, The AVI Publishing Company Inc., U.S.A.

All units should be expressed in metric system. Figures in the text should be given Arabic numerals and their position appropriately marked in the text. All figures should have captions. Tables (as few as possible) should be typed on separate paper and should be given Arabic numerals and their position marked in the text. Each table should have a caption.

For every contribution the author will be supplied fifteen gratis reprints in the case of single author and ten reprints each in the case of joint authorship. Number of extra copies required (with or without cover) at cost price plus postage must be communicated to the Editor, on receipt of communication regarding acceptance of the paper for publication.

The number of words per article should be restricted to 3,000 for review articles, 2,000 for research papers and 1,000 for notes.

Statement about ownership and other particulars about the newspaper (journal)

“FISHERY TECHNOLOGY”

(To be published in the first issue every year after the last day of February)

(Form IV)

(See Rule 8)

- | | |
|-------------------------------|--|
| 1. Place of publication | : Cochin-682 029 |
| 2. Periodicity of publication | : Six monthly |
| 3. Printer's name | : N. Unnikrishnan Nair |
| Nationality | : Indian |
| Address | : Society of Fisheries Technologists (India)
Matsyapuri P. O., Cochin-682 029 |
| 4. Publisher's name | : N. Unnikrishnan Nair |
| Nationality | : Indian |
| Address | : Society of Fisheries Technologists (India)
Matsyapuri P. O., Cochin-682 029 |
| 5. Editor's name | : N. Unnikrishnan Nair |
| Nationality | : Indian |
| Address | : Society of Fisheries Technologists (India)
Matsyapuri P. O., Cochin-682 029 |

I, N. Unnikrishnan Nair, hereby declare that the particulars given above are true to the best of my knowledge and belief.

Cochin-682 029
1-7-1981

Sd/-
N. Unnikrishnan Nair,
Editor & Publisher

Oxidation of Residual Lipids in Fish Protein Concentrates and its Effect on the Nutritional Quality of the Protein

K. DEVADASAN

Central Institute of Fisheries Technology, Cochin-682 029

The paper reviews the work reported on the changes in the nutritive value of fish protein concentrates (FPC) during storage, with special emphasis on the effects of the interactions between oxidised residual lipids and proteins of the FPC. Theories on the oxidised lipid-protein interactions are reviewed and the nutritional significance of these reactions is discussed.

Fish protein is fast gaining acceptance as the final answer to the problem of protein malnutrition in developing nations. With the alarming growth of population in many of these countries, the demand for high quality protein is increasing very fast. The hitherto under utilised marine protein resources alone can meet this demand. Developed nations, especially the Scandinavian countries, are supplying fish protein concentrates (FPC) to developing nations for fighting protein malnutrition. This non-defatted fish protein concentrate from lean fishes, supplied for human consumption, is often referred to as FPC type B. In India also inspite of the traditional vegetarian food habits among sizeable sections of the population, fish protein is becoming acceptable. When FPC is intended for human consumption its nutritional quality is to be studied more thoroughly. There are various factors affecting the nutritional quality of FPC, among which oxidation of residual lipids during storage of FPC is perhaps the most important. This paper reviews the studies on this important aspect of fish protein utilization.

Fish meal and FPC change in colour and odour during storage. These changes are more pronounced when the storage temperature is high. In tropical climate, FPC thus develops undesirable colour and odour faster than in temperate climates. These changes are known to be related to the oxidation of the polyunsaturated fatty acids present in the residual lipids of FPC.

Laksheswari & Aga (1965) undertook a detailed study on the effect of prolonged

storage of FPC on its quality. The study was conducted with winter herring fish meal stored in paper bags, for 12 years. Studies by Carpenter (1960) indicated a regular reduction in fluorodinitrobenzene (FDNB) available lysine during storage of FPC for 12 years. But Ambrose & Synder (1964) observed that the *invitro* pepsin digestibility of protein is not affected by storage. When used as the sole source of protein in diets for chicks, the protein quality as determined by protein efficiency ratio (PER) did not change in 7 years, after which it showed signs of deterioration. But the same meal when used as a supplement to a cereal diet, did not show any deterioration on storage.

Solvent extracted FPC generally shows lesser reduction in protein quality during storage than FPC samples from which fat is not removed completely.

Oxygen availability and temperature of storage are two important factors affecting the protein quality in stored non-defatted FPC. According to March *et al.* (1961) low temperatures do not necessarily prevent loss in protein quality. However many other studies have indicated that low temperature storage, especially in the absence of oxygen has a favourable effect on protein quality. Lea *et al.* (1958) compared the protein quality of a sample of herring meal stored at 25°C in presence of air, with a sample stored at -20°C under nitrogen atmosphere. With progressive storage, FDNB lysine registered a greater decrease in the sample stored at 25°C in presence of air. But a similar sample when solvent extracted

to remove fat prior to storage at 25°C, retained the content of FDNB lysine comparable to the control sample. Lea *et al.* (1960) confirmed these observations in a subsequent study. But an interesting observation made was that though FDNB lysine was more in the sample kept at freezing temperatures under N₂ atmosphere, it did not show a corresponding better protein quality in feeding trials. Decline in quality as judged by chemical indices (FDNB lysine, available methionine, *in vitro* pepsin digestibility) and PER was observed by Carpenter *et al.* (1963) in FPC stored for one year at 20°C with access to air. Miller *et al.* (1970) studied the effect of storage temperature on the quality of FPC by feeding trials, using chicks. During a two year period of storage, the decline in growth promoting quality was similar for two samples of menhaden meal, one stored at -20°C and the other at 25°C. They observed no change in FDNB lysine content during storage but pepsin digestibility showed a gradual decrease.

The influence of the species of fish used for making FPC on the reduction in quality during storage was studied by March *et al.* (1966). In a comparison between herring and menhaden meals they did not observe any significant difference between the FPC samples. In their experiments on chick growth with meals, all samples showed a decline in quality in the first 6-11 months, after which the changes were not significant.

The effect of lipid oxidation during storage of FPC, on its protein quality, has been the subject of many investigations in different laboratories.

Njaa *et al.* (1966) made detailed studies using fish meal prepared from different species of herring. The samples were stored with and without addition of butylated hydroxy toluene (BHT) and the effect of lipid oxidation on the nutritive value was studied by feeding trials on rats. The values were compared with those for freshly prepared fish meals. These studies could not demonstrate a significant reduction in protein quality induced by fat oxidation. However the reduction in protein quality due to lipid oxidation was apparent in meals prepared from certain species like the North

Sea herring, though in Scando-Atlantic herring meals, this effect was not striking. The fact that BHT added meals also showed some reduction in protein quality suggests that antioxidants cannot arrest these deteriorative changes completely.

Opstvedt *et al.* (1970) and Opstvedt (1975) have reported the effect of lipid oxidation on the protein quality of fish meal. In these studies ethoxyquin is used (1, 2 dihydro-6-ethoxy, 2, 2, 4 trimethylquinoline) is used as antioxidant to prevent lipid oxidation. Commercially prepared fish meal samples from North Sea herring and mackerel were stored with and without addition of ethoxyquin for a period of 5 years. The changes in protein quality as measured by PER, NPU (net protein utilization) and available methionine and lysine, showed that the antioxidant added sample was 15% better than the controls. The deteriorative changes in the samples were apparent during the first year of storage after which the decrease in quality was negligible. Studies conducted by El-Lakany & March (1974) in Canada on the effect of lipid oxidation on protein quality of fish meal have also lead to some interesting conclusions. They used herring meal for the study which was stored at 20°C with and without addition of ethoxyquin. One sample was stored at -20°C without addition of antioxidant also. After 40 weeks of storage, the samples were compared to evaluate their protein quality. In FDNB reactive lysine, *in vitro* pepsin digestibility and the ability to support growth in chicks, there was no significant difference between the sample stored at -20°C without antioxidant and that stored at 20°C with antioxidant. However the samples stored at 20°C without antioxidant was inferior in quality. Studies by March *et al.* (1965) showed that meals containing oxidised fat supported 6-9% lower growth in chicks compared to unoxidised meals, when fed as a supplement to a cereal diet, though the two samples were comparable in their FDNB reactive lysine.

South African workers have reported results of similar studies on pilchard meal. A comparison of pilchard meals stored with and without addition of ethoxyquin did not show any consistent effect of storage time on the protein quality. However the

antioxidant added meal was superior in its FDNB reactive lysine, available methionine, protein digestibility as well as NPU when compared to the control sample (Dreosti *et al.* 1969). Wessels (1971) and Wessels & Moodie (1975) found inconsistent and variable differences between antioxidant treated and untreated South African anchovy meals after storage. They tested the samples by feeding chicks. Almquist (1956) did not observe any reduction in pepsin digestibility in anchovy meal after 4 months storage. But storage for another 7 months caused a 2% reduction.

In India Moorjani *et al.* (1965) studied the changes in protein quality of air dried anchovy meal, with normal fat content, during storage at 28–33°C for 2 months. They have reported a 15% reduction in FDNB reactive lysine in these samples compared to controls from which fat was removed by solvent extraction prior to storage. There was no significant difference in the PER values of the samples when tested on rats. But the fat containing sample was found to be inferior in its pepsin digestibility and available essential amino acids.

Geisler & Contreras (1967) investigated the effect of increased fat in a fish meal sample on its protein quality during storage. They added increasing amounts of anchovy oil (0–15%) to solvent extracted anchovy meal and kept in petridishes for 5 months. With increasing oil content, there was a corresponding decrease in protein digestibility and FDNB reactive lysine. These changes were more pronounced during the first 15 days after which further changes were less apparent.

Thus it is seen that during storage of non-defatted FPC, there is a noticeable loss in its nutritional quality. This deterioration can be retarded by the addition of an antioxidant and by keeping the FPC in an inert atmosphere. Removal of fat by solvent extraction also retards the deteriorative changes. These observations suggest that the loss in nutritional quality of the protein is related to the oxidation of residual lipids in the FPC and the interaction of the protein with oxidised lipids.

A review of the studies and theories on the reaction of protein and oxidised lipids is therefore desirable. Several reviews covering different aspects of these interactions are available (Kaunitz, 1967; Schauenstein, 1967; Tappel, 1973; Karel *et al.*, 1975; Gardner, 1979).

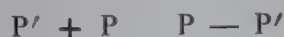
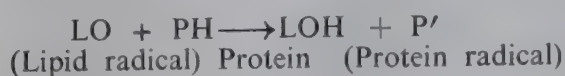
For studying the protein-oxidised lipids reactions, Tappel (1955) made use of a model system in which emulsions of linoleic acid or cod liver oil were oxidised at 37°C for 24 h in an aqueous suspension of casein and other proteins. The studies suggested a denaturation of proteins by oxidised lipids. Both the protein and lipid fractions were found to lose their characteristic chemical properties. Tappel (1955) therefore suggested the formation of co-polymers between proteins and the oxidation products from lipids. He postulated covalent linkages between the carbonyls from oxidised lipids and the amino groups of proteins, similar to those formed during Maillard reaction between proteins and reducing sugars. Later studies (Venolia & Tappel, 1958) could not however confirm this. But chemical bonds between oxidized lipids and protein were observed by Desai & Tappel (1963). They found that peroxy bonds are the main bonds formed during this reactions.

Narayan & Kumerow (1958) reported results of similar studies. Narayan *et al.* (1964) postulated the formation of a net work of hydrogen bonds holding the oxidised lipids between parallel peptide chains of proteins, yielding an extended beta keratin configuration. Cumulatively the weak hydrogen bonds can give stability to the lipids protein complex. This theory assumes that the ketonic and hydroxy groups are the main reactive groups involved in protein-oxidised-lipid interactions and that the functional amino and sulphhydryl groups are not involved in linkages.

Kwon *et al.* (1965) and Crawford *et al.* (1967) observed that proteins interact with aldehydes, products of oxidative degradation of lipids, forming enamine bonds. The bifunctional malonaldehyde can cross link protein chains via schiff base formation. This aldehyde has therefore received special attention in such studies. Chio & Tappel

(1969) studied reactions of malonaldehyde and protein and suggested intra and inter molecular cross binding. Reactions of malonaldehyde and amino acids have been studied by Buttkus (1969) also.

Raubal & Tappel (1966) postulated that protein polymers can be formed without direct participation of lipids and that the lipid fraction only initiates the polymerisation reactions. Lipid fractions of the protein lipid co-polymers could either be embedded in the protein polymer or connected to the protein polymer by non-covalent linkages. The reactions according to this theory, could be represented as



These authors suggested formation of disulphide bonds in proteins forming such polymers. But Zirlin & Karel (1969) have suggested that reactions between oxidized lipids and proteins may lead to breakage of the protein polypeptide chain, especially at low water activity.

Thus it can be seen that various types of reactions are possible in the interaction between oxidized lipids and proteins. They can be classified into four general types.

- i) Protein polymers without any lipids
- ii) Polymers of proteins and oxidized lipids
- iii) Co-valently linked polymers of oxidized lipids and proteins
- iv) Polymers of oxidized lipids and broken peptide chains

The relative preponderance of each of these in the product may depend on various factors like reaction conditions, nature of the lipid and protein. Of these, the polymers held together by physical bonds require water for their stability and so cannot be expected to be present in systems like FPC type B which has a low moisture content.

It is known that polymers held by co-valent linkages are favoured by acidic conditions, whereas alkaline conditions favour formation of physical bonds. Temperature also has an effect on this reaction rate as shown by Venolia & Tappel (1958) and Kwon *et al.* (1965). They showed that an increase in temperature brings about a corresponding increase in the reaction rate between oxidised lipids and proteins. However these reactions could proceed even at a very low temperature (Braddock & Dugan, 1973). Lea *et al.* (1960) also observed an increase in reaction rate with temperature though this effect was less pronounced beyond 37°C. Yanagita *et al.* (1973) have also supported this view. But Buttkus (1967) found some other physical conditions, besides temperature, also equally important. He showed that the reaction between protein and malonaldehyde was almost the same at -20°C and 20°C even though this was considerably higher than that at 0°C. He attributes this effect of freezing to a concentration of reactants and also to a catalytic action of ice crystals. It is reasonable to think that temperature affects the rate and not the type of reaction.

The effect of the lipid on the interaction between proteins and oxidised lipids was studied by Yanagita *et al.* (1973) and Giesler and Contreras (1967). They observed that increased lipid in the protein, increased the interactions though this is disputed by some workers.

These studies were all with dry proteins. In aqueous systems, the rate of reaction between oxidised lipids and proteins varied depending on the nature of the protein as shown by Venolia & Tappel (1958) and Raubal & Tappel (1966).

The picture of the interactions between auto oxidising lipids and proteins is thus a complex one. It is generally believed that the lower aldehydes formed are the most reactive compounds in such a system. The low flavour threshold values of lower aldehydes also makes them very important compounds in these studies (Sessa & Rackis, 1977; Eriksson *et al.* 1976). Hydroperoxides are more reactive with proteins than ketones. In the protein part, the active site can be the free amino group of lysine or guanidyl group of arginine. The free

carbonyl group of acidic amino acids also may react with hydroperoxides forming ester bonds. —SH group of cysteine, S—S—group of cystine and sulphide group of methionine are also reactive.

Relatively few studies have been reported on the effect of the interactions between oxidised lipids and proteins on the nutritional availability of proteins. Most of the reported results are based on *in vitro* studies. Desai & Tappel (1963) studied the effect of these interactions on the rate of hydrolysis of the protein by dilute hydrochloric acid. The native protein was hydrolysed more easily, compared to the protein-oxidized lipid complex. The rate of hydrolysis showed maximum reduction with histidine, serine, proline, arginine and the sulphur amino acid residues.

Raubal & Tappel (1966) indicated that the deciding factor was the nature of the protein. Buttkus (1967) reported the temperature dependence of the reaction. At 100°C the amino acids mostly affected were histidine, arginine, tyrosine and methionine in that order, whereas at frozen temperatures the losses were maximum for lysine, tyrosine, methionine and arginine in that order. Braddock & Dugan (1973) observed considerable loss in total histidine, lysine and methionine when linoleate was oxidised in a solution of myosin at 50°C. Studying the reaction between malonaldehyde and albumin in an aqueous medium. Crawford *et al.* (1967) found out that FDNB reactive lysine registered a regular decrease with increasing malonaldehyde concentration. Pepsin digestibility also decreased when oxidised lipids reacted with proteins (Yanagita *et al.*, 1973; El. Lakany & March, 1974).

In *in vivo* tests, it is generally seen that proteins after reaction with oxidised lipids, take longer time to leave the stomach, that too in a comparatively lesser degraded state. But in PER and in intestinal digestion no adverse effect is observed. Heating of protein in presence of oxidised lipids is found to reduce protein digestibility in general. All amino acids are generally affected by this, but the effect is more pronounced in lysine and methionine. Oxidative changes in methionine is particularly easy. Methionine is oxidised to its sul-

phoxide, which further gets oxidised to methionine sulphone. But this takes place in acidic conditions only (Njaa, 1962) and so in natural foods this does not easily happen (Cuq *et al.* 1973; Slump & Schreuder, 1973). Cystein, the other sulphur containing amino acid is oxidized to cysteic acid when casein is heated with oxidized lipids (Tannenbaum *et al.* 1969). But this reaction is not very fast at room temperature. Tannenbaum *et al.* (1969) has correlated the oxidation of methionine to the polymerization of protein. Many authors maintain, that methionine sulphone and cysteic acid cannot serve as sources for the parent amino acids in diets. (Njaa, 1962; Miller and Samuel, 1970). However methionine sulphoxide is reported to have the same biological activity as DL-methionine, though D-methionine sulphoxide is found to have reduced biological activity (Njaa, 1962, Slump & Schreuder, 1973). But Miller & Samuel (1970) and Ellinger & Palmer (1969) report results contrary to this. According to them DL-methionine sulphoxide has only 25% biological activity of DL-methionine. Cuq *et al.* (1973) and Kuzmicky *et al.* (1974) support this view. These differences may be due to different factors. Rats may adapt to utilize methionine sulphoxide or utilization of methionine sulphoxide may improve with age. Again the free methionine sulphoxide and methionine sulphoxide in a protein may not be utilized to the same extent. Recent reports also suggest that presence of oxidized sulphuramino acids in the protein chains makes it resistant to hydrolysis, reducing the utilization of all amino acids (Pieniazek *et al.* 1975). The studies in general have one thing in common. While protein digestibility as such may fall only marginally due to reactions between oxidised lipids and proteins, the loss in availability of individual amino acids is much greater. This is an aspect which has not been satisfactorily explained so far. It can be said that, lysine and the sulphur containing amino acids are affected to a greater extent by these interactions. Histidine and tyrosine are also among the amino acids easily damaged by oxidised lipids.

The oxidised lipid-protein interactions is thus a complex problem, which calls for much more detailed study to arrive at clear and definite conclusions.

References

- Almquist, H. J. (1956) *Agric. Fd Chem.* **4**, 638
- Ambrose, M. & Synder, D. G. (1964) *Fishg. News int.* **3**, 210
- Braddock, R. J. & Dugan, L. R. Jr. (1973) *J. Am. Oil Chem. Soc.* **50**, 343
- Buttkus, H. (1967) *J. Fd Sci.* **32**, 432
- Buttkus, H. (1969) *J. Am. Oil Chem. Soc.* **46**, 88
- Carpenter, K. J. (1960) *Biochem. J.* **77**, 604
- Carpenter, K. J., Lea, C. H. & Parr, L. J. (1963) *Br. J. Nutr.* **17**, 151
- Chio, K. S. & Tappel, A. L. (1969) *Biochem.* **8**, 2827
- Crawford, D. L., Yu, T. C. & Sinnhuber, R. O. (1967) *J. Fd Sci.* **32**, 332
- Cuq, J. L., Provansal, M., Gillux, F. & Cheftel, C. (1973) *J. Fd Sci.* **38**, 11
- Desai, I. D. & Tappel, A. L. (1963) *J. Lipid Res.* **4**, 204
- Dreosti, G. M., Merve, R. P. & Post, B. J. (1969) *Fishing Industry's Research Institute (FIRI South Africa) Annual Report* p. 40
- El-Lakany, S. & March, B. E. (1974) *J. Sci. Fd Agric.* **25**, 899
- Ellinger, G. & Palmer, R. (1969) *Proc. Nutr. Soc.* **28**, 42 A
- Eriksson, C. E., Lundgren, B. Vallentin, K. (1976) *Chem. Senses Flavour.* **2**, 3
- Gardner, H. W. J. (1979) *J. Agric. Fd Chem.* **27** (2), 220
- Geisler, H. W. & Contreras, E. (1967) *Fishg. News int.* **6**, 38
- Karel, M., Schaich, K. & Roy, R. B. (1975) *J. Agric. Fd Chem.* **23**, 159
- Kwon, T. W., Menzel, D. B. & Olcott, H. S. (1965) *J. Fd Sci.* **30**, 808
- Kuzmicky, D. D., Kohler, G. V., Walker, H. G. Jr. & Mackey, B. E. (1974) *Poult. Sci.* **53**, 45
- Kaunitz, B. H. (1967) *Fd Technol.* **21**, 278
- Lakesvela, B. & Aga, A. T. (1965) *J. Sci. Fd Agric.* **16**, 743
- Lea, C. H., Parr, L. J. & Carpenter, K. J. (1958) *Br. J. Nutr.* **12**, 297
- Lea, C. H., Parr, L. J. & Carpenter, K. J. (1960) *Br. J. Nutri.* **14**, 91
- March, B. E., Biely, J., Goudie, C., Claggott, F. & Tarr H. L. A. (1961) *J. Am. Oil Chem. Soc.* **38**, 80
- March, B. E., Biely, J., Tarr, H. L. A. & Claggott, F. (1965) *Poult. Sci.* **44**, 679
- March, B. E., Biely, J., Goudie, C. & Tarr, H. L. A. (1966) *J. Fish. Res. Bd Can.* **23**, 395
- Miller, D., Kifer, R. R. & Ambrose, M. E. (1970) *Poult. Sci.* **49**, 1005
- Miller, D. & Samuel, P. D. (1970) *J. Sci. Fd Agric.* **21**, 616
- Moorjani, M. N., Lahiri, N. L., Balakrishnan Nair, R., Upadhye, A. N. & Venkat Rao, S. (1965) *Fd Technol.* **19**, 110
- Narayan, K. A. & Kumerow, F. A. (1958) *J. Am. Oil Chem. Soc.* **35**, 52
- Narayan, K. A., Sugai, M. & Kumerow, F. A. (1964) *J. Am. Oil. Chem. Soc.* **41**, 254
- Njaa, L. R. (1962) *Br. J. Nutr.* **16**, 571
- Njaa, L. R., Utne, F. & Braekkan, O. R. (1966) *Fiskeri, dir. Skr. Ser. Technol. Unders.* **5**, 1
- Opstvedt, J. Olsen, S. & Urdahl, N. (1970) *Acta. Agric. Scand.* **20**, 175
- Opstvedt, J. (1975) *Acta Agric. Scand.* **25**, 53
- Pienizek, D., Radowska, M. & Kunachowicz, H. (1975) *Br. J. Nutr.* **34**, 163

- Raubal, W. T., Tappel, A. L. (1966) *Arch. Biochem. Biophys.* 113
- Schauenstein, E. (1967) *J. Lipid Res.* 8, 417
- Sessa, D. J., Rackis, J. J. (1977) *J. Am. Oil Chem. Soc.* 54, 468
- Slump, P., Schreuder, H. A. W. (1973) *J. Sci. Fd Agric.* 24, 657
- Tannenbaum, S. R., Barth, H. & Rowx, J. P. L. (1969) *J. Agric. Fd Chem.* 17, 1353
- Tappel, A. L. (1955) *Arch. Biochem. Biophys.* 54, 266
- Tappel, A. L. (1973) *Proc. Fed. Am. Soc. Exp. Biol.* 32, 1870
- Venolia, A. W. & Tappel, A. L. (1958) *J. Am. Oil Chem. Soc.* 35, 135
- Wessels, J. P. H. & Moodie, T. (1975) *J. Sci. Fd Agric.* 26, 625
- Wessels, J. P. H. (1971) *Agronomalia.* 3, 125
- Yanagita, T., Sugano, M., Cho, S. & Wada, M. (1973) *J. Agric. Chem. Soc. Japan* 47, 99
- Zirlin, A. & Karel, M. (1969) *J. Food Sci.* 34, 160

Biology of Fouling in Neendakara Port, a Tropical Estuary in the South West Coast of India

K. DHARMARAJ and N. BALAKRISHNAN NAIR

Department of Aquatic Biology and Fisheries, University of Kerala, Trivandrum-695007

Species composition and some aspects of the biology of the fouling community in Neendakara port (southwest coast of India) has been examined for a period of one year. Fouling organisms were collected with a system of glass panels exposed for varying durations and during different months in the port. One species of sponge, nine species of coelenterates, thirteen species of polyzoans, four species of mud-tube dwelling polychaetes, four species of serpulids, one species each of mud-tube forming amphipod and tanaid, two species of oysters, six species of mussels and not less than eight species of tunicates were the macro foulers which settled over the panels. Monthly and seasonal settlement of the different species have been recorded. Fouling has been a continuous process occurring throughout the year in Neendakara port with slightly fluctuating biomass and considerably varying species composition. Alternate species dominance of marine and brackish water forms has been an important feature of fouling in the area. Number of species of the sedentary fouling animals represented on test panels has been high during the highly saline premonsoon period and low during the monsoon period.

Interest in the problem of marine fouling brings together operators of vessels, naval architects, harbour engineers, anti-fouling paint manufacturers and all those concerned with the maintenance of ships and various underwater installations. Diverse talents of biologists, chemists, physicists and engineers are required for a thorough understanding and effective control of fouling. However, basic knowledge indispensable for any advanced study of the problem and to formulate effective preventive measures is the biology of the organisms concerned with fouling, because fouling is a biological phenomenon resulting from the settlement and growth of animals and plants over submerged objects. The severity of the problem of fouling can be realised from the recent estimate that in the United States, military and commercial marine interests suffer an annual loss of about 500 million dollars owing to marine fouling, without including indirect disadvantages such as higher fuel costs and increased frequencies of dry docking (Persoone, 1977). Nearly a decade ago, it was estimated that the mechanised fishing crafts of India alone would require approximately 4.5 million rupees annually to keep their

hulls free from fouling and to have a smooth sailing, that too, for a limited period only (Balasubramanyan *et al.*, 1972). The magnitude of the problem can only be roughly predicted since estimates covering all aspects of loss owing to fouling are very scarce and are extremely difficult to obtain.

Fouling community includes sessile or sedentary representatives from almost all the invertebrate phyla and a few of the lower chordate divisions. Besides, many free living organisms are also found among the sedentary representatives. The structure and composition of the fouling community exhibit wide temporal and regional variations governed mainly by varying hydrographical conditions and geographical location. More than 1400 species of animals have been identified from the fouling complex before 1952 (Anon, 1952) and since then also several species have been continuously added on to the list, but an exhaustive recent review is lacking. The fouling fauna of Indian navigational waters and ports have been inadequately investigated (Karande, 1978) and earlier works pertain to fouling in the harbours or ports at Bombay (Karande, 1968 a, b), Goa (Dehadri *et al.*,

1975; Harkantra *et al.*, 1977), Mangalore (Menon *et al.*, 1977), Cochin (Nair, 1967; Nair, 1967; Balasubramanyan & Nair, 1970; Menon & Nair, 1971; Santhakumari & Nair, 1975), Visakhapatnam (Ganapati & Rao, 1968; Ganapati *et al.*, 1958; Rao & Ganapati, 1978), Madras (Raja, 1959, 1963, Daniel, 1954, 1955), Port Blair (Karande, 1978) and Tuticorin (Renganathan *et al.* in press). With the advent of the culture of oysters and mussels along the coasts of India, the impact of fouling on mariculture has been realised to some extent (Kuriyan, 1950; Harkantra *et al.*, 1977) and the impediments of fouling to pearl oyster culture have also been briefly reported (Alagaraswamy & Chellam, 1976).

The present paper represents a part of the results obtained during the course of a detailed study on the marine fouling and timber destroying organisms carried out in Neendakara port situated at the mouth of the Asthamudi backwaters in the south-west coast of India. The objectives of the study are 1. to identify the major fouling animals of the port, 2. to determine the nature and extent of fouling, 3. to determine the relative importance of various species of animals in the fouling community, 4. to find out the period of settlement of the different species, 5. to record the seasonal variations in the nature and intensity of fouling, and 6. to obtain the nature of influence of environmental variables on fouling.

Materials and Methods

Fouling organisms were collected by exposing glass panels of 10 x 10 cm size with one smooth and one rough surface, submerged and held in position at about 1 m below the lowest low water mark in wooden racks suspended from a fixed jetty beam. Individual panels were identified by labelled plastic tags and all possible contacts of the panels among them and with supporting structures were avoided. Three series of test panels were exposed simultaneously from February 1977 to January 1978 as in Fig. 1.

A-series (short-term)

Twelve panels exposed one by one at the beginning of a month and changed at the

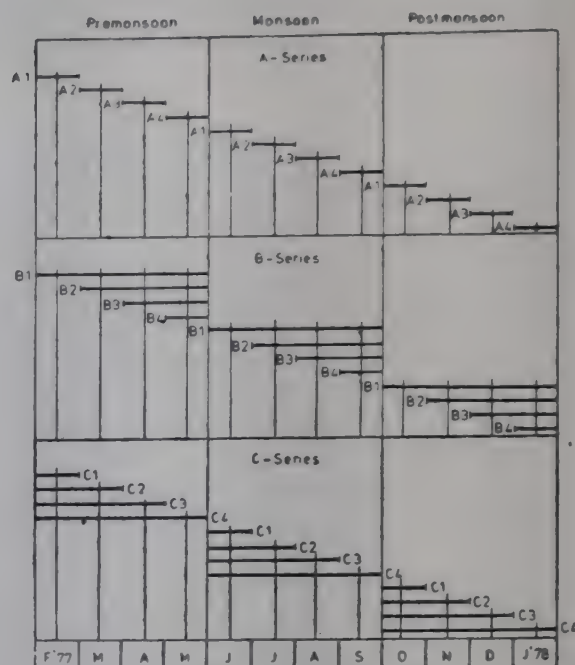


Fig. 1. Plan of test panel exposures

beginning of the succeeding month formed this series. This showed the settlement which had taken place during the period of immersion and gave an indication of the monthly variations of settlement on panels qualitatively and quantitatively.

B-series (long-term)

To obtain data regarding the seasonality in the settlement and rate of increase in the biomass of fouling, the year was divided into three periods namely the premonsoon (February to May), the monsoon (June to September) and the postmonsoon (October to January). At the beginning of every month panels were immersed and they were collected simultaneously at the end of each period. Thus, three sets of B-series panels, each set consisting of four panels were exposed for durations ranging from one to four months (Fig. 1) and examined.

C-series (long-term)

Three sets each consisting of four panels were exposed in the beginning of each period and raised one by one at the end of each month, after exposing them for durations ranging from one to four months during

each period. This series gave an idea of the fouling settlement for the respective period of immersion, growth of the community in terms of biomass and indicated how the monthly settlement was modified by the foulers already present on the panels.

The panels with the settled fouling elements were preserved in formalin after determining the total biomass of the community. The foulers were identified upto species level as far as possible and for colonial forms the relative abundance of the different species was determined. In the case of solitary forms which could be counted separately, the exact number of individuals of the different species has been determined.

The locality

The exposure tests were carried out in Neendakara port (Lat. $08^{\circ}30'N$; Long. $76^{\circ}53.3'E$) which is a typical estuarine habitat situated at the mouth of the Ashtamudi backwaters having a permanent connection with the adjoining Arabian sea lying on the west. This is an important fishing harbour of the southwest coast of India, visited by and providing landing jetties to a large number of trawlers daily. The fishing craft of the area include mechanised vessels and canoes. The mouth of this estuary is wide and deep and therefore, the influence of the sea is strongly felt inside the port throughout the year. During the monsoon period, influx of fresh water into the backwater and its outflow through the gut is considerable. From June to September there is heavy rainfall from the southwest monsoon and from October to December there is varying quantities of precipitation from the northeast monsoon. Based mainly on the quantity of rainfall received during different months, the year could be divided into three seasons as stated earlier, even though this division is arbitrary. Usually, the premonsoon is the hot and highly saline period during which the experimental site experiences hydrographic conditions very similar to that of the nearby coastal marine environment. The monsoon period is characterised by almost fluviatile conditions with fluctuating low salinity and temperature and the postmonsoon period represents the transitional stage between the other two. However, during

the course of the present study, the southwest monsoon burst over the region during the later half of May with moderate rainfall, which continued during the postmonsoon period also consequent on the precipitation from the northeast monsoon.

Wave action inside the port is considerably reduced as a result of the artificial breakwaters which shelter the port. The rocky and concrete boulders of the breakwaters, the pillars of jetties, and other submerged objects in the neighbourhood, provide excellent substrata for the settlement and growth of a variety of epibenthic fauna, which supply larvae to foul freshly submerged objects and the hulls of fishing craft. Height of the water column at the experimental site reaches a maximum of about 4 m depending on the monsoonal floods, and tidal ebb and flow. Throughout the year, movements of fishing boats and other vessels, and tidal current churn up the water leaving fair amounts of silt to settle over the panels.

Results and Discussion

Ecology

Salient features of the different ecological variables in the port which have been discussed in detail earlier (Dharmaraj & Nair, 1979, 1981) are summarised here. Salinity of surface water remained high during the first three months of the premonsoon period (29.0 to 32.5‰) with an increasing trend from February to April and in May it declined to 26.5‰, but that of the bottom water remained above 31.5‰ throughout this period (Fig. 2). During the monsoon period low salinity prevailed and the lowest value (13.7‰) was recorded during September in the surface water. In the bottom water also salinity declined from June to September to reach the minimum (16.5‰) value. Low saline conditions continued till December during the postmonsoon period with slight fluctuations, and the values again rose up by January to reach 31.5 and 32.4‰ in the surface and bottom waters respectively. Salinity was always found to be high at the bottom and the vertical stratification was more pronounced during the rainy season.

The premonsoon period was characterised by high temperature which showed a rising trend from February to April till it reached the peak value recorded, (32.0°C) both at the surface and at the bottom (Fig. 2). With the onset of the southwest monsoon, temperature started falling and the lowest values in the surface and the bottom waters (26.5 and 26°C) were recorded in July and September respectively. Temperature fluctuated between 29.3 and 30.5°C at the surface and 29.2 and 30°C at the bottom during the postmonsoon period. Thermal stratification of the water column varied irregularly with warmer waters at the surface except in July and the stratification was insignificant during the premonsoon period.

Dissolved oxygen of the surface water varied from 4 to 4.6 ml/l, whereas the same of the bottom water ranged between 3.6 and 4.0 ml/l. A definite pattern of seasonal change could not be noticed in the dissolved oxygen, but always a vertical gradient existed with low values at the bottom. Light attenuation coefficients calculated from Secchi disc readings of light penetration depths (Fig. 2), indicate that the water at the experimental site had been highly turbid during most

parts of the year. *K*-values below one has never been obtained and the range was between 1.11 and 4.83. The water was found to be comparatively less turbid during the premonsoon period than during the other two periods.

Composition of the fouling community

Major sedentary animals of the fouling community at Neendakara port consisted of sponges, hydroids, sea anemones, polychaetes, serpulids, cirripedes, oysters, mussels and tunicates. The species composition and certain aspects of the ecology of the macro sedentary animals alone are reported in this paper. Mud-tube forming polychaetes, amphipods and tanaids are also included since they contribute much to fouling. The nature of settlement of the different species of fouling animals over the short-term (A-series) and long-term (B & C series) panels is presented in Tables 1 to 3. Porifera contributed a species of sponge and coelenterates were represented by not less than eight species of hydroids and a species of sea anemone. Diversity of bryozoans in the fouling community was high with as many as thirteen estuarine and marine species. Four species each of serpulids and mud-tube forming polychaetes are recorded. The amphipod *Corophium triaenonyx*, the tanaid *Tanais estuarius* and the cirripede *Balanus amphitrite communis* were the dominant fouling crustaceans of the port. Several other species of free living amphipods, isopods and other groups were also found amidst the bush fouling over the panels. Bivalves were represented by two species of oysters and nearly six species of mussels. One species of simple ascidian and not less than seven species of compound ascidians were noticed in the fouling community, but specific identifications of these posed difficulties and so their cumulative abundance alone is recorded.

Monthly and seasonal settlement of fouling animals

Sponges appeared rarely on the short-term panels during the premonsoon period (Table 1). On the long-term panels (B & C-series) they were very common on those

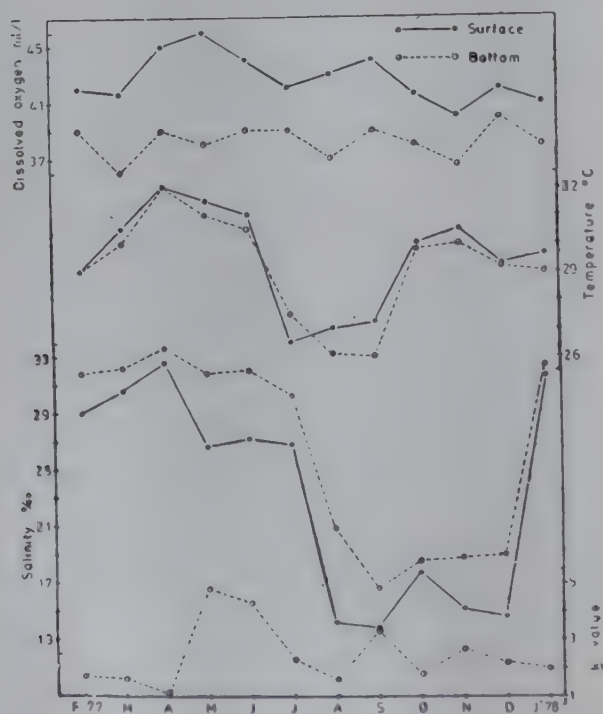


Fig. 2. Monthly variations of environmental facts at the test site

Table 1. Monthly settlement of major sedentary fouling organisms (A-series)

Sedentary fouling organisms	Period of submergence												1978
	1977	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan
Surface area													
SPONGES	S	R	S	R	S	R	S	R	S	R	S	R	S
COELENTERATES	—	—	—	R	—	—	—	—	—	—	—	—	—
<i>Obelia bicuspidata</i>	A	A	A	A	A	C	—	—	R	C	VC	C	VC
<i>Clytia</i> sp.	VR	VR	R	—	—	—	—	—	—	—	—	—	—
<i>Bimberia vesitita</i>	C	VC	C	C	MC	VR	R	—	—	—	—	R	C
<i>Halocordyl edisticha</i>	C	C	A	A	A	C	—	—	—	—	—	C	R
<i>Eudendrium</i> sp.	R	R	C	C	C	—	—	—	—	—	—	—	—
<i>Garveia</i> sp.	R	R	C	C	—	—	—	—	—	—	—	—	R
<i>Ectopleura</i> sp.	—	—	—	C	R	R	—	R	—	—	R	—	R
<i>Sea anemones</i>	9	35	12	27	4	13	—	—	2	1	3	3	7
POLYZOANS	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Electra crustulenta</i>	—	—	—	—	—	R	C	C	R	R	VC	R	R
<i>Electra bengalensis</i>	—	—	R	MC	—	—	—	—	—	—	—	MC	R
<i>Alderina arabianensis</i>	VC	A	C	VC	VC	A	VC	—	—	—	—	R	C
<i>Bugula neritina</i>	R	R	MC	MC	—	R	—	—	—	—	—	—	C
<i>Bugula cucullata</i>	C	VC	A	A	A	—	—	—	VR	R	VC	—	R
<i>Schizoporella cochinensis</i>	C	C	C	VC	C	A	MC	VC	—	—	—	—	R
<i>Schizomavella linearis</i>	R	R	VR	MC	MC	VR	—	—	—	—	—	—	R
<i>Watersipora subovoidea</i>	R	C	MC	C	MC	VC	—	—	—	—	—	—	R
<i>Victorella pavidia</i>	—	—	—	—	—	—	C	VC	A	R	VC	R	C
<i>Vesicularia papuensis</i>	—	—	R	MC	—	—	—	—	—	—	—	—	—
<i>Bowerbankia gracilis</i>	—	—	R	MC	C	—	—	—	—	—	—	—	—
MUD-TUBE DWELLING POLYCHAETES	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Eunice</i> sp.	6	8	4	7	2	8	—	—	—	2	2	8	5
<i>Polydora</i> sp.	—	—	—	—	—	—	3	6	5	7	2	4	6
<i>Pectinaria</i> sp.	—	—	—	—	—	—	2	4	3	1	1	—	—
<i>Branchiomma</i> sp.	13	16	9	7	3	5	—	—	—	2	2	9	4
SERPULIDS	—	—	—	—	—	—	—	—	—	—	—	1	5
<i>Hydroides brachicantha</i>	7	16	8	14	13	15	3	6	—	3	5	2	6
<i>Mercierella enigmatica</i>	—	—	—	—	—	—	—	—	—	—	—	3	7
<i>Pomatoleios croslandi</i>	17	53	13	25	7	12	—	—	8	12	3	7	3
<i>Ficopomatus macrodon</i>	—	—	—	—	—	—	2	6	15	18	6	16	15
	—	—	—	—	—	—	—	—	13	5	16	3	5

(Contd.)

Table 1. (Contd.)

MUD-TUBE FORMING
AMPHIPODS & TANAIIDS

<i>Corophium triaenonyx</i>	14	17	9	12	12	16	17	23	31	40	67	85	14	26	7	16	9	13	14	35	23	45	16	21
<i>Tanais estuarius</i>	—	—	—	—	—	—	3	4	2	5	9	6	13	17	24	18	3	6	14	12	3	—	15	11

CIRRIPEDES

<i>Balanus amphitrite communis</i>	—	—	9	16	13	18	23	31	21	45	29	73	4	97	35	56	9	13	35	87	17	33	—	3
------------------------------------	---	---	---	----	----	----	----	----	----	----	----	----	---	----	----	----	---	----	----	----	----	----	---	---

BIVALVES

<i>Anomia</i> sp.	—	3	1	2	—	4	—	—	—	—	—	1	—	—	—	—	—	—	—	—	2	—	—	3
<i>Crassostrea madrasensis</i>	1	2	3	5	9	14	1	4	—	—	2	6	17	24	2	1	—	—	—	—	1	4	2	9
<i>Perna indica</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	2	8	—	—	—	—	—	—
<i>Perna viridis</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	2	6	4	—	—	2	5	—	—	—	—
<i>Modiolus carvalhoi</i>	4	3	3	6	2	7	4	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Modiolus plumicens</i>	—	—	2	6	5	7	3	8	—	—	2	—	—	—	—	—	—	2	—	1	—	—	—	—
<i>Musculista senhousia</i>	1	4	2	3	4	6	7	4	2	9	3	5	4	8	5	13	—	—	—	3	5	9	2	13
<i>Musculista arcuata</i>	—	—	—	—	—	—	—	—	—	—	—	—	5	6	2	4	5	3	2	7	4	3	4	7

TUNICATES

Simple ascidians	—	—	—	—	2	3	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Compound ascidians	C	VC	MC	C	R	R	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	R	MC	—

Key: S = smooth; R = rough; A = abundant; VR = very rare; MC = moderately common; C = common; VC = very common; — = absent

Table 2. Settlement of major fouling organisms on long term (B-series) panels

Sedentary fouling organisms	1977												Period of submergence												1978																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																			
	Feb-May						Mar-May						Apr-May						May						Jun-Sep						July-Sep						Aug-Sep						Sep-Jan						Oct-Jan						Nov-Jan						Dec-Jan						Jan																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																									
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R

(Contd.)

Table 2 (Contd.)

SERPULID POLYCHAETES

<i>Hydroides brachiacantha</i>	15	23	17	24	12	30	3	6	3	5	5	7	4	7	—	8	21	12	16	8	11	3	12
<i>Mercierella enigmatica</i>	—	—	—	—	—	—	—	—	11	83	10	14	9	16	9	12	14	24	9	12	3	3	—
<i>Pomatolictos crostlandi</i>	12	25	17	23	7	11	—	—	—	—	—	—	—	—	—	26	47	33	40	29	37	15	48
<i>Ficopomatius macrodon</i>	—	—	—	—	—	—	—	—	7	31	20	33	6	12	6	13	13	18	12	17	9	13	—

MUD-TUBE FORMING
AMPHIPODS & TANAIDS

<i>Corophium triaenonyx</i>	3	9	2	7	—	—	17	23	50	77	40	45	29	36	7	16	17	26	14	22	9	15	16	21
<i>Tanais estuarius</i>	2	6	—	—	—	—	3	4	21	29	25	31	17	28	24	18	2	1	10	19	12	16	15	11

CIRRIPEDES

<i>Balanus amphitrite communis</i>	35	53	26	31	10	18	23	31	107	139	98	130	77	95	35	56	49	108	20	31	10	13	—	3
BIVALVES	—	2	2	4	3	4	—	—	—	—	—	—	—	—	—	—	1	3	—	2	1	—	—	3
<i>Crassostrea madrasensis</i>	5	8	7	9	4	6	1	4	11	24	13	24	9	17	2	1	2	3	7	9	3	5	2	9
<i>Perna indica</i>	—	—	—	—	—	—	—	—	2	4	—	6	—	2	—	4	—	—	—	—	—	—	—	—
<i>Perna viridis</i>	—	—	—	—	—	—	—	—	3	8	3	—	1	4	6	4	—	3	—	4	—	6	—	—
<i>Modiolus carvolhoi</i>	8	9	14	17	3	6	4	6	—	—	—	—	—	—	—	—	4	7	4	7	—	2	—	—
<i>Modiolus plumicens</i>	6	13	3	9	2	5	3	8	—	—	—	—	—	—	—	—	3	8	5	6	2	1	—	—
<i>Musculista senhousia</i>	18	35	12	16	3	7	7	4	19	36	21	34	12	18	5	13	11	23	20	31	3	7	12	13
<i>Musculista arcuata</i>	—	3	—	—	—	—	—	—	10	17	12	26	14	30	2	4	—	—	2	8	3	6	4	7

TUNICATES

Simple ascidians	1	4	6	9	2	7	1	—	—	—	—	—	—	—	—	—	2	3	2	4	3	4	—	—
Compound ascidians	A	A	C	VC	R	MC	—	—	R	R	—	—	R	R	—	—	VC	A	VC	A	VC	A	R	MC

Key: S=smooth; R=rare; VR=very rare; R=very rare; MC=moderately common; C=common; VC=very common; A=abundant; —=absent

Table 3. *Settlement of major sedentary fouling organisms on long-term (C-series) panels*

[illegible]

(Contd.)

Table 3 (Contd.)
SERPULID POLYCHAETES

<i>Hydroides brachiacantha</i>	7	16	13	16	9	17	15	23	—	—	—	—	—	—	3	5	3	5	8	11	12	15	8	21
<i>Mercierella enigmatica</i>	—	—	—	—	—	—	—	—	—	2	7	12	13	18	11	23	3	7	12	27	10	17	14	24
<i>Pomatoleios croslandi</i>	17	53	16	26	14	29	12	25	—	—	—	—	—	—	—	—	—	—	9	13	23	36	26	47
<i>Ficopomatus macrodon</i>	—	—	—	—	—	—	—	—	2	6	8	11	15	24	7	31	3	5	8	17	12	16	13	18

MUD-TUBE FORMING
AMPHIPODS & TANAIDS

<i>Corophium triaenonyx</i>	14	17	4	10	13	14	3	9	31	40	43	59	54	73	50	77	19	13	13	24	27	43	17	26
<i>Tanais estuarius</i>	—	—	—	—	3	2	2	6	2	5	12	17	14	23	21	29	3	6	7	12	13	18	2	1

CIRRIPEDES

<i>Balanus amphitrite communis</i>	—	—	17	28	26	39	35	53	21	45	82	115	96	133	107	139	9	3	42	94	54	102	49	108
------------------------------------	---	---	----	----	----	----	----	----	----	----	----	-----	----	-----	-----	-----	---	---	----	----	----	-----	----	-----

BIVALVES

<i>Anomia</i> sp.	—	3	—	2	1	4	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	2	1	3
<i>Crassostrea madrasensis</i>	1	2	3	6	1	9	5	8	—	—	14	23	12	20	11	24	—	—	—	2	1	4	2	3
<i>Perna indica</i>	—	—	—	—	—	—	—	—	—	—	—	—	1	5	2	4	2	8	—	2	—	—	—	—
<i>Perna viridis</i>	—	—	—	—	—	—	—	—	—	—	—	2	5	7	3	8	—	—	4	5	2	3	—	—
<i>Modiolus carvolhoi</i>	4	3	5	8	8	12	8	9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	7
<i>Modiolus plumicens</i>	—	—	7	11	3	9	6	13	—	—	—	—	—	—	—	—	—	2	—	—	1	4	3	8
<i>Musculista senhauisia</i>	1	4	5	12	13	27	18	35	2	9	7	13	21	39	19	36	—	—	4	9	12	18	11	23
<i>Musculista arcuata</i>	—	—	—	—	—	—	—	3	—	—	2	6	12	14	10	17	5	3	3	8	2	4	—	—

TUNICATES

Simple ascidians	—	—	—	3	8	4	1	4	—	—	—	—	—	—	—	—	—	—	—	—	2	1	2	3
Compound ascidians	C	VC	MC	C	C	A	A	A	—	—	—	—	—	—	R	R	—	—	—	MC	C	VC	UC	A

Key: S = smooth; B = rough; VR = very rare; MC = moderately common; C = common; VC = very common; A = abundant; — = absent

exposed for four months during the premonsoon period (Tables 2 & 3). The settlement of sponges was poor during the postmonsoon period and it was totally absent during the monsoon period.

Among the hydroids, *Obelia bicuspidata* was abundant during the premonsoon and common during the postmonsoon months with no evidence of settlement from June to August. The pattern of its settlement was almost similar over the long-term panels also, with increasing density corresponding to the duration of exposures. Over the long-term series, *Obelia gracilis* also appeared during the pre and postmonsoon periods. The incidences of other hydroids such as *Clytia* sp., *Bimeria vestita*, *Halocordyl edisticha*, *Eudendrium* sp. and *Garveia* sp. were also intense during highly saline summer months. Some of them appeared in lesser abundance during the postmonsoon months also, and the representation of *Ectopleura* sp. was erratic. In general, hydroids were represented very poorly during the monsoon period. Sea anemones were abundantly noticed on the short-term panels submerged during the pre and postmonsoon periods. On the long-term panels also, abundant settlement of sea anemones was discernible during the pre and postmonsoon periods. They were found on all the four panels of the B-series and the longest submerged panels of the C-series in lesser numbers during the monsoon period.

Electra crustulenta and *Victorella pavidia* were the brackish water polyzoans which fouled the panels considerably during the monsoon and postmonsoon periods. Both of them did not appear during the premonsoon period. *Bugula cucullata*, *Schizoporella cochinesis* and *Schizomavella linearis* were common during the postmonsoon and very common during the premonsoon periods. *Aetea anquina* and *Averrilla setigera* were found only on the longterm panels. *Electra bengalensis* appeared rarely on the short-term panels and commonly on the long-term panels during the premonsoon period. *Alderina arabianensis*, *Bugula neritina* and *Watersipora subovoidea* were also very commonly found on all the three series of panels during the pre-monsoon period. *Vesicularia papuensis* and *Bowerbankia gracilis* appeared erratically during the pre-

monsoon period only. In general, fouling by bryozoans were heavy during the premonsoon, moderate during the post monsoon and poor during the monsoon periods if the two brackish water species were excluded.

Polychaetes such as *Eunice* sp., *Polydora* sp., *Pectinaria* sp. and *Branchiomma* sp. assisted towards the accumulation of significant quantities of mud over the panels. Even though some of the above mentioned forms are errant, they are sluggish and were living on the panels almost like attached forms. Over the short-term panels, *Eunice* sp. and *Branchiomma* sp. were invariably noticed during the pre and postmonsoon periods. *Polydora* sp. and *Pectinaria* sp. were abundant during the monsoon period, the former extending its settlement to the postmonsoon period too. The nature of the settlement of these polychaetes was almost similar on the long term panels also, however, with increasing numbers with the duration of exposure during the monsoon and postmonsoon periods. In the case of the settlement of *Branchiomma* sp. over the premonsoon C-series panels, maximum number was recorded on one month panel than over the panels exposed for two to four months.

Serpulid polychaetes encountered during the present study were *Hydroides brachiacantha*, *Mercierella enigmatica*, *Pomatoleios croslandi* and *Ficopomatus macrodon*. *Hydroides brachiacantha* fouled the A-series panels during the pre and postmonsoon periods. Over the B-series panels, its incidence was noticed during the monsoon period also though poorly. Fouling by this species exhibited a similar trend over the C-series panels with heavy incidence during the pre and postmonsoon periods. *Pomatoleios croslandi* appeared densely during the first three months of the premonsoon and last two months of the postmonsoon periods on the short-term panels. Settlement of this species was heavy during the postmonsoon period on the B-series panels and during the premonsoon on the C-series. *Mercierella enigmatica* and *Ficopomatus macrodon* were not found during the premonsoon period over any of the series. These two species appear to be brackish water forms settling densely during the monsoon and postmonsoon periods.

Mud accumulating crustaceans such as the amphipod *Corophium triaenonyx* and the tanaid *Tanais estuarius* also heavily fouled the panels of all the three series. *C. triaenonyx* was present throughout the year with peak incidence during the monsoon and *T. estuarius* was collected during the monsoon and postmonsoon months from the A-series. Over the B-series, settlement of *C. triaenonyx* was dense during the monsoon period and over this series, its incidence was poor during the premonsoon on the panels exposed for two to four months. The nature of the settlement of *T. estuarius* on the B-series panels was similar to that on the A-series, however, with more numbers. C-series also exhibited the same pattern of fouling by these two species as over the A and B-series.

The most dominant fouling crustacean from the Neendakara port was the cirripede *Balanus amphitrite communis* and it appeared throughout the year on all the series except during February when it was absent on the A-series. The peak months of its settlement were August and November on the A-series. The nature of its settlement over the B-series indicated that it has heavily fouled the panels during the monsoon and the beginning of the postmonsoon periods. C-series also exhibited a similar pattern of fouling by this barnacle.

Bivalves came next in abundance to barnacles in the fouling community, represented among others by *Anomia* sp. and *Crassostrea madrasensis*. *Anomia* sp. was rare sporadically over all the series of panels. On the A-series, *C. madrasensis* settled densely during August and April. On the B-series, settlement of *C. madrasensis* was discernible over all the panels, however, it was done over the panels exposed during the monsoon period. C-series panels also showed heavy fouling by *C. madrasensis* during the monsoon period. Two species of mussels namely *Perna indica* and *P. viridis* appeared erratically on the panels and the nature of their settlement suggests that they settle during the later half of the monsoon and the first half of the postmonsoon periods.

The bivalves which contributed substantially to the bulk of fouling were four species of mussels namely *Modiolus carvolhoi*, *M.*

plumicens, *Musculista senhausia* and *M. arcuatula*. On the A-series, the first two of these mussels appeared only during the premonsoon in considerable numbers. *M. senhausia* settled almost throughout the year and *M. arcuatula* fouled the panels during the postmonsoon and the later half of the monsoon periods. The pattern of fouling by these mussels over the B-series indicated that *M. carvolhoi* and *M. plumicens* settle densely during the premonsoon with some incidence during the postmonsoon months. Over, all of the B-series panels, fouling by *M. senhausia* was discernible with erratic heavy settlement. *M. arcuatula* was abundant during the monsoon period. The nature of the settlement of these mussels over the C-series also was in similar pattern.

Simple ascidians appeared during April and May over the A-series and their settlement over the long-term panels was erratic with no sign of settlement during the monsoon period. Compound ascidians were poorly represented on the A-series appearing only during the highly saline period. Over the B and C-series, the settlement of compound ascidians was dense with not less than seven species during both the pre and post-monsoon periods.

Biomass of the fouling community

Over the A-series maximum biomass (72.8 g) was recorded during September and the minimum (16.9 g) during October (Fig. 3). The biomass was considerably high during the premonsoon and monsoon months and low during the postmonsoon period over the short-term panels. The nature of accumulations for four months over the B-series (Fig. 3) indicates that, among the three periods, the premonsoon recorded the maximum value (170.5 g), the monsoon the lowest (144.8 g) and the postmonsoon the intermediate (159.3 g). But, in three month exposures, post monsoon panels registered the lowest (112.6 g), monsoon the intermediate (123.3 g) and premonsoon the highest (142.0 g) biomass values. The pattern over the two month exposure panels was also similar to that of the three month exposures.

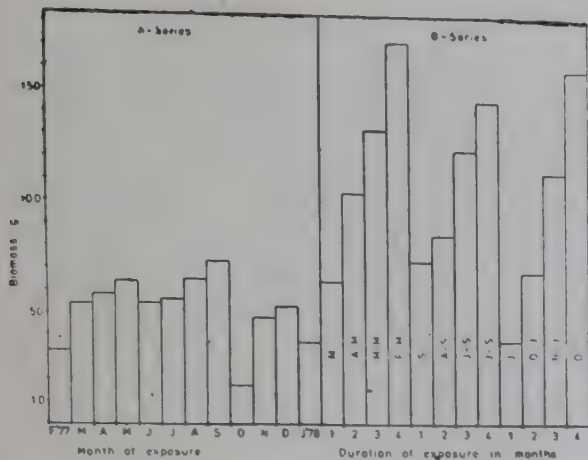


Fig. 3. Biomass of fouling over the A & B series test panels

In the C-series, the biomass accumulations over the four month panels were identical to those of the B-series. On three month panels, maximum biomass was recorded during the premonsoon period (165.9 g), minimum during the monsoon period (129.5 g) and intermediate value (136.8 g) during the postmonsoon period (Fig. 4). A similar pattern was noticed on the two month panels also.

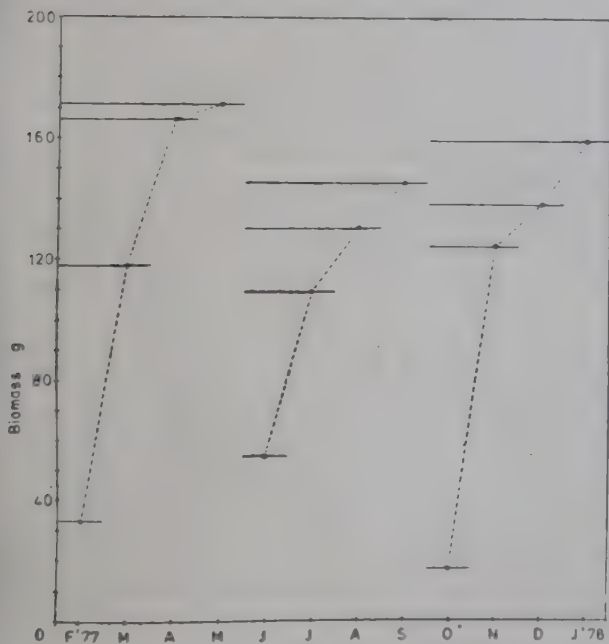


Fig. 4. Rate of increase of fouling biomass during different seasons (C-series)

The present study reveals that the settlement of fouling organisms at the Neendakara port is a continuous process occurring throughout the year with fluctuating densities and varying species composition. The diverse nature of fouling and the dominance of different groups of organisms are illustrated in Figs. 5 to 8. Although certain dominant species settle almost continuously, the density of their settlement significantly varies during different months and seasons. Many species show a regular seasonal pattern of settlement comparable with the reports from other estuarine regions of India (Menon & Nair, 1971; Nair, 1967). There is fairly heavy fouling during all the months over the short-term panels. The biomass of fouling over the long-term panels indicate a constant growth of the already settled fouling community, along with the fresh recruitment depending upon the reproductive characteristics of the concerned species.

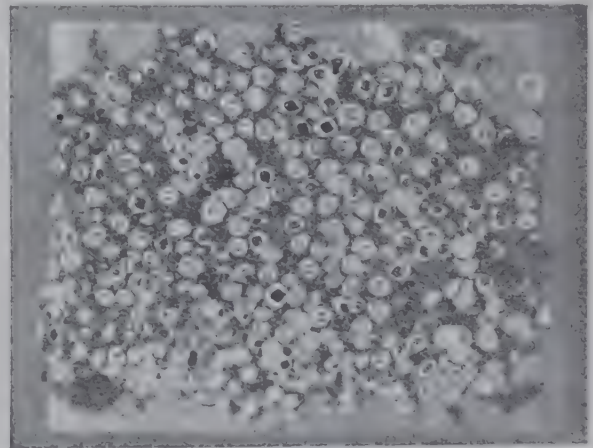


Fig. 5. Photograph of the fouling community dominated by barnacles on the smooth surface of a panel

In general, most of the foulers settled more densely on the rough surfaces of the panels than over the smooth surfaces, the former being more favourable for securing a foothold for the free swimming larvae under a constantly moving medium. However, in some instances greater settlement of certain selected species of foulers was, in fact, noticed over the smooth surface than on the rough surface.

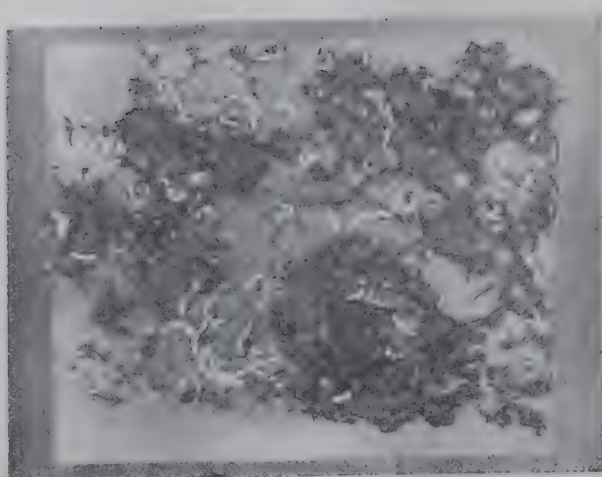


Fig. 6. Fouling community composed of serpulids, mussels and an oyster

An obvious and important feature of fouling at Neendakara port is the alternate seasonal dominance of marine and estuarine forms in the community. During the premonsoon period, coastal marine sedentary organisms abundantly settle and dominate, during the monsoon period typical estuarine forms dominate and during the postmonsoon period representatives from both these forms are noticeable. All the nine species of coelenterates encountered during the present study can be considered marine forms occurring densely during the premonsoon period. Among the thirteen species of fouling bryozoans, only *Electra crustulenta* and *Victorella pavida* were able to survive and occur in abundance during the monsoon period and these two species were absent during the premonsoon period. All the other bryozoans were more common during the premonsoon than during the other periods and may, therefore, be considered as marine.

Polydora sp. and *Pectinaria* sp. are estuarine forms which fouled the panels in large numbers during the monsoon period and *Eunice* sp. and *Branchiommata* sp. are marine forms settling densely during the pre and postmonsoon periods. Among the serpulid polychaetes, *Mercierella enigmatica* and *Ficopomatus macrodon* seem to be restricted to low saline period while *Hydroides brachiacantha* and *Pomatoleios croslandi* seem to prefer highly saline periods. Polychaete fouling was rich and varied during

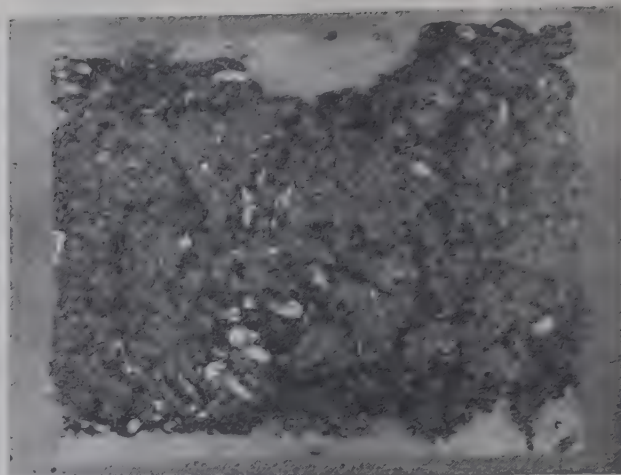


Fig. 7. Heavy fouling with mud matted over the panels by mussels, mud-tube dwelling amphipods and polychaetes

the postmonsoon owing to the settlement of both estuarine and marine forms. The contribution of the edible mussels *Perna indica* and *P. viridis* to the fouling was considerable only over the long-term panels exposed during the monsoon and postmonsoon periods. Among the other mussels, *Musculista arcuatula* is an estuarine form fouling densely during the monsoon and postmonsoon and *Modiolus carvolhoi* and *M. plumicens* are marine forms which preferred highly saline periods for their dense settlement.

Only three species, namely *Balanus amphitrite communis*, *Corophium triaenonyx* and *Musculista senhausia* have been found to foul the panels almost throughout the year. By virtue of its dense settlement during certain period and the almost continuous nature of its presence, the barnacle *Balanus amphitrite communis* may be considered as the most dominant of the fouling animals at the Neendakara port. Barnacles have been reported to have different peak periods of settlement in different localities of India (Menon *et. al.* 1977). The continuous breeding activity of barnacles (Pillai, 1958; Nair, 1967) and the consequent supply of larvae for recruitment throughout the year would have been responsible for the fouling by barnacles recorded over almost all the panels.

Major factors that could influence the quality and quantity of fouling at Neenda-

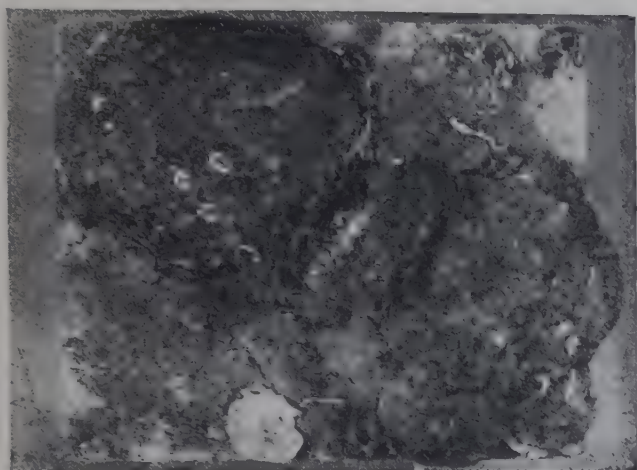


Fig. 8. Fouling by oysters, barnacles and serpulids with the dominance of oysters

kara port are 1. salinity structure and other hydrographical conditions, 2. movements of fishing and other vessels into and out of the port, 3. reproductive periodicities of the endemic epibenthic fauna, and 4. inter-specific and intraspecific competition among the foulers. During the premonsoon period, larvae of the epibenthic fauna of the rocky boulders of the breakwaters facing the Arabian sea and the pillars of the Neendakara bridge find their way to the experimental site through tidal and other water movements. During this period almost a typical littoral epibenthic faunal assemblage fouls the panels. This faunal assemblage is nearly eliminated during the monsoon period when brackish water species dominate in the fouling complex with lesser species diversity. The larvae of the brackish water forms could be recruited from the endemic epibenthic fauna of the piles and laterite banks of the upper reaches of the Ashtamudi backwaters.

The existence of certain species of mussels, bryozoans, serpulids and other fouling organisms in the backwater during the monsoon period when the salinity is greatly reduced may be due to long-term physiological adjustment and gradual acclimatisation. Among the several species dominant during the premonsoon period, only very few which can tolerate low saline conditions settle and survive along with other brackish water forms during the monsoon period. The postmonsoon period represents a transitional stage between the other two periods

with regards to hydrographical conditions and fouling during this period also has been of intermediary nature. The mesohaline conditions prevailing during the postmonsoon period allow the brackish water representatives of the fouling community to survive until salinity increases beyond their limits of tolerance during the premonsoon period.

Species diversity of the fouling community of Neendakara port has been high during the pre and postmonsoon periods and low during the monsoon period. This is due to the incidence of a large number of species of hydroids and bryozoans during high saline periods. Salinity seems to play the major role in regulating the species abundance in the fouling community. However, fouling biomass has been little affected by the species abundance, because many of the species found to dominate during the monsoon period are larger forms such as mussels and oysters which contribute substantially to the biomass. Besides, during the monsoon period, accumulation of mud by mud-tube forming amphipods, tanaid and polychaetes has also been intense along with the natural siltation over the panels. These factors are responsible for the heavy biomass of fouling recorded during the monsoon period when the species diversity of the fouling community was low.

The authors wish to express their gratitude to Dr. N. R. Menon for the identification of bryozoans and acknowledge the financial assistance received from the University Grants Commission, New Delhi.

References

- Alagaraswamy, K. & Chellam, A. (1976) *Indian J. Fish.* 23, 10
- Anon (1952) *Marine Fouling and its Prevention*, Woods Hole Oceanographic Institution, Woods Hole, United States Naval Institute, Maryland, U. S. A., 388 pp.
- Balasubramanyan, R. & Nair, N. U. (1970) *Proc. Symp. Mollusca, Mar. Biol. Ass. India.* 3, 730
- Balasubramanyan, R., Nair, N. U. & Pillai, A. G. G. K. (1972) *3rd Int. nat.*

- Cong. Mar. Corr. Fouling*, Gaithersburg, Maryland, U. S. A., p. 898
- Daniel, A. (1954) *J. Madras Univ.* **24** B, 189
- Daniel, A. (1955) *J. Madras Univ.* **25** B, 189
- Dehadri, P. V., Parulekar, A. H. & Untawale, A. G. (1975) *J. Bombay nat. Hist. Soc.* **72**, 580
- Dharmaraj, K. & Nair, N. B. (1979) *Aqua. Biol.* **4**, 49
- Dharmaraj, K. & Nair, N. B. (1981) *All India Seminar on the Status of Environmental Studies in India, Trivandrum*, p. 27 (Abstract)
- Ganapati, P. N. & Rao, K. S. (1968) *Curr. Sci.* **37**, 81
- Ganapati, P. N., Rao, M. V. L. & Nagabhushanam, R. (1958) *Andhra Univ. Mem. Oceanogr.* **62**, 193
- Harkantra, S. N., Nair, Z. A., Ansari, A. & Parulekar, A. H. (1977) *Mahsagar, Bull. natn. Inst. Oceanogr.* **10**, 165
- Karande, A. A. (1968 a) *Def. Res. Dev. Org. Rep. India*, 1
- Karande, A. A. (1968 b) *2nd Int. nat. Cong. Mar. Corr. Fouling*, Athens, Greece, p. 563
- Karande, A. A. (1978) *Indian J. mar. Sci.* **7**, 39
- Kuriyan, G. K. (1950) *J. Bombay nat. Hist. Soc.* **49**, 90
- Menon, N. R. & Nair, N. B. (1971) *Mar. Biol.* **8**, 280
- Menon, N. R., Katti, R. J. & Shetty, H.P.C. (1977) *Mar. Biol.* **41**, 127
- Nair, N. B. (1967) *Proc. Symp. Crustacea, Mar. Biol. Ass. India.* **4**, 1254
- Nair, N. U. (1967) *Hydrobiologia*: **30**, 180
- Persoone, G. (1977) *Applications for Copper in the Mariculture Industry, A review. INCRA Research Report*, 41 pp.
- Pillai, N. K. (1958) *Bull. Cent. Res. Inst., Univ. Kerala*, **6**, 117
- Raja, B. T. A. (1959) *J. Mar. Biol. Ass. India*, **1**, 180
- Raja, B. T. A. (1963) *J. Mar. Biol. Ass. India*, **5**, 113
- Rao, K. S. & Ganapati, P. N. (1978) *Proc. Indian Acad. Sci.* **87** B, 63
- Renganathan, T. K., Nair, N. B. & Dharmaraj, K. *Indian J. Mar. Sci.* (In Press)
- Santhakumari, V. & Nair, N. B. (1975, *Bull. Dept. Mar. Sci., Univ. Cochin*) **7**, 827

The Influence of Plating Technique and Incubation Temperature on Bacterial Count from Fish and Fishery Products

NIRMALA THAMPURAN, H. KRISHNA IYER and K. MAHADEVA IYER
Central Institute of Fisheries Technology, Cochin-682 029

For bacterial sampling of raw unprocessed fish and frozen fishery products, spread plate method is preferable to pour plate method; incubation of plates at 30°C gives a higher count than incubation at 37°C. Analysis of variance of the data shows that sample variation between different types of fishes is highly significant whereas the variations between triplicate plates is not significant at 5% level.

Increasing attention is paid nowadays to the microbiological quality of food-stuffs and this necessitates a thorough study of the techniques adopted for assessing their microbial content. Total aerobic bacterial count (TABC) or total plate count (TPC) is the most important means of establishing whether a product has been prepared under strict sanitary condition.

Different techniques are in use for determining the TPC of which the spread plate technique and the pour plate technique are the most common and widely accepted. Pour plate technique involves the use of melted agar maintained at 45°C, while spread plate technique employs pre-set plates of agar equilibrated to the desired temperature.

The ISI method for the bacteriological examination of fresh fish as well as frozen fish products stipulates that pour plate technique be used along with an incubation temperature of 37°C (IS: 1977 a, b). The same procedure is also suggested for microbiological analysis of shell fish and its products (AOAC, 1970).

There are several reports on the role of transient warm agar temperature in decreasing the microbial recoveries by pour plate technique (Zobell & Conn, 1940; Stapert *et al.* 1962; Van Soestbergen & Lee, 1969; Klein & Shenyu Wu, 1974). Majority of the marine micro-organisms are found to succumb when exposed to a temperature of 30-40°C for 10 min. Recently several communications have come forth, which

claim spread plate method to be superior to pour plate method especially in the case of seafoods (Lee & Harward, 1970; Nottingham *et al.* 1975; Shenyu Wu & Klein, 1976). On the other hand Mossel & Van der Moosdijk (1963) found no difference between pour plate (agar 44-46°C) and spread drop plate (agar at room temperature) of 42 food samples.

A study was undertaken in this laboratory to find out the suitability of these two procedures for use in bacteriological sampling of fish and fishery products and to evaluate their performance.

Materials and Methods

Raw fish samples were procured either from local country crafts or landing centres near Cochin. Samples were analysed within 2h after collection. Raw fish samples analysed were in very fresh condition as judged by their appearance. 39 samples of raw fish and 48 samples of frozen materials were studied during 1976-79. Frozen samples were obtained from a local seafood processing plant and hence of unknown history. The frozen blocks were kept in cold storage till required. Muscle homogenates of the raw or frozen samples were prepared by grinding about 10 g of the material with 90 ml of normal saline in a sterile mortar. Subsequent dilutions were prepared in the same diluent by mixing 1 ml of the sample dilution with 9 ml of the diluent in a vortex mixer. Sea water agar (SWA) for sampling raw fish and tryptone glucose agar (TGA) for frozen material were used. The agar

media for spread plating had been poured 6 h before the experiment and excess moisture evaporated by keeping the plates at 45°C for one hour. Care was taken to prevent syneresis fluid on the agar surface to assure that colony spreading would be minimised. 0.5 ml of the appropriate dilutions were spread evenly on the agar surface with an alcohol flamed bent glass rod. Pour plating was done using 1 ml of the dilution. The molten agar was maintained at 45°C in a thermostatic water bath. All samples were plated in triplicate. One set of plates from each method was kept at room temperature, ($29 \pm 1^\circ\text{C}$; RT) and another at 37°C. In addition, a set of spread plates was also kept at 8°C for psychrotrophic count. RT and 37°C counts were taken at the end of 48 h of incubation and low temperature count after three weeks.

Results and Discussion

Average counts from three spread plates and three pour plates were used for statistical analysis. For raw minced fish, the microbial count varied from $8.4 \times 10^4/\text{g}$ of the muscle to $1.3 \times 10^6/\text{g}$ (Table 1.) The maximum count was noted in spread plates incubated at room temperature. The lowest count was obtained by pour plating and incubating at 37°C. For frozen products the count varied from $3 \times 10^3/\text{g}$ of the material to $1.2 \times 10^6/\text{g}$ (Table 2). In frozen and raw fish, spread plates incubated

at room temperature gave highest count and pour plates at 37°C the lowest. The count at 8°C was used to estimate the prevalence of psychrotrophic bacteria in the sample. Analyses of variance for different fishes are presented in Tables 3 to 10.

From Table 3, it is found that for sardines (*Sardinella longiceps*) there is no significant difference in bacterial count between temperatures. But difference existed between methods, spread plate count being greater than pour plate count. The analysis of variance (Table 4) for mullets (*Mugil cephalus*) showed significant difference in bacterial count between temperatures, RT giving highest count than 37°C. However there was no significant difference with methods. In silver jew fish (*Johnius dussumieri*) and Indian halibut (*Psettodes erumei*) the trend was similar (Tables 5 and 6). The variation in bacterial count between temperatures as well as methods were highly significant. Room temperature and spread plates yielded higher bacterial counts.

Regarding frozen sardines, significant difference in count was observed between the two temperatures as well as the two methods (Table 7). RT and spread plates consistently gave higher counts. Fillets of frozen jew fish (*Pseudosciaena* sp.) and the mixed fish muscle paste popularly known as 'Kheema' (Tables 8 and 10) showed more or less similar behaviour. But for fillets of cat fish (*Tachysurus* sp.) differences

Table 1. Total bacterial count for raw fish (total plate count/g $\times 10^4$)

	(29 \pm 1)°C		37°C		8°C
	S.P.	P.P.	S.P.	P.P.	S.P.
Sardine	230*	190	200	203	—
Sardine	6812	3110	1900	1400	48
Sardine	1275	594	4300	3400	3005
Sardine	17	21	20	27	13
Mullet	170	780	85	91	68
Mullet	93	781	8	8	—
Mullet	540	87	96	160	—
Indian halibut	13100	13100	12000	11000	6000
Silver jew fish	742	306	290	280	102

* Average of three values

S.P.—Spread plate method
P.P.—Pour plate method

existed only between the two temperatures, RT giving higher bacterial count than 37°C (Table 9).

In all, sample variation was highly significant, but variation between triplicate plates was not significant at 5% level. A similar finding was also reported by Nottingham *et al.* (1975).

For raw fish as well as frozen samples, room temperature gave higher count than 37°C. The only exception was raw sardines for which the count variation due to temperature difference was not very much marked. Identical counts obtained at RT and 37°C for sardine can be accounted by the fact that the samples had been exposed to ambient temperature for a longer time. Jan Gjerde (1975) had reported that the higher count at 37°C for raw fish can be considered as an indication that the fish has been exposed to higher temperatures, since normally psychrotrophic bacteria predominate in raw fish. It has also been suggested that a comparison of count at 25 and 37°C gives a measure of relative proportion of psychrotrophs and mesophiles (Nottingham *et al.* 1975).

Table 2. *Total bacterial count for frozen fish (total plate count/g $\times 10^3$)*

	(29 \pm 1)°C		37°C		8°C
	S.P.	P.P.	S.P.	P.P.	S.P.
	g	g	g	g	g
Sardine	21*	6.7	13	7.6	—
Sardine	180	109	160	108	4.6
Sardine	181	76	74	68	37
Sardine	65	46	56	32	3.2
Sardine	210	150	190	65	—
Sardine	21	9	16	8	—
Jew fish	46	19	36	11	14
Jew fish	57	81	39	48	21
Cat fish	1200	638	530	570	30
Cat fish	188	170	108	77	—
Cat fish	314	206	29	20	8
Kheema	449	317	278	242	11

*Average of three values

S.P— Spread plate method

P.P— Pour plate method

Table 3. *Analysis of variance for raw sardine*

Source	S.S.	df	MS	F
Total	47.9681	71	—	—
Samples	44.1282	5	8.8256	188.18 ^c
Temperature	0.0040	1	0.0040	0.085
Methods	0.8828	1	0.8828	18.82 ^c
Triplicate	0.0002	2	0.0001	0.002
Error	2.9529	63	0.0469	—

c — Significant at 0.1% level

Table 4. *Analysis of variance for raw mullet*

Source	S.S.	df	MS	F
Total	20.4909	59	—	—
Samples	9.1154	4	2.27885	15.45 ^c
Temperature	3.6608	1	3.6608	24.819 ^c
Methods	0.1937	1	0.1937	1.313
Triplicate	0.0003	21	0.00015	1
Error	7.5207	51	0.1475	—

c — Significant at 0.1% level

Table 5. *Analysis of variance for raw Indian halibut*

Source	S.S.	df	MS	F
Total	0.0119	11	—	—
Temperature	0.0094	1	0.0094	67.14 ^c
Methods	0.0012	1	0.0012	8.57 ^a
Error	0.0013	9	0.00014	—

a, c – Significant at 5 and 0.1 % respectively

Table 6. *Analysis of variance for raw silver jew fish*

Source	S.S.	df	MS	F
Total	0.3691	11	—	—
Temperature	0.1483	1	0.1483	13.12 ^b
Methods	0.1188	1	0.1188	10.51 ^a
Error	0.1020	9	0.0113	—

a, b – Significant at 5 and 1 % respectively

Table 7. *Analysis of variance for frozen sardine*

Source	S.S.	df	MS	F
Total	11.8636	71	—	—
Samples	9.9333	5	1.98666	179.46 ^c
Temperature	0.2351	1	0.23510	21.24 ^c
Methods	1.0061	1	1.00610	90.89 ^c
Triplicate	0.0027	2	0.000135	21
Error	0.6864	62	0.01107	—

c – Significant at 0.1 % level

Table 8. *Analysis of variance for frozen jew fish*

Source	S.S.	df	MS	F
Total	1.6288	23	—	—
Samples	0.7593	1	0.7593	27.51 ^c
Temperature	0.2280	1	0.2280	8.26 ^a
Methods	0.1450	1	0.1450	5.25 ^a
Triplicate	0.0003	2	0.0002	—
Error	0.4962	18	0.0276	—

a, c – Significant at 5 and 0.1 % respectively

Table 9. *Analysis of variance for frozen cat fish*

Source	S.S.	df	MS	F
Total	9.5148	35	—	—
Samples	5.8035	2	2.9028	63.80 ^c
Temperature	2.2526	1	2.2526	49.51 ^c
Methods	0.1383	1	0.1383	3.04
Triplicate	0.0002	2	0.0001	1
Error	1.3182	29	0.0435	—

c – Significant at 0.1 % level

Table 10. *Analysis of variance for frozen kheema*

Source	S.S.	df	MS	F
Total	0.1191	11	—	—
Temperature	0.0793	1	0.0793	113.29 ^c
Methods	0.0334	1	0.0334	47.71 ^c
Error	0.0064	9	0.0007	—

c – Significant at 0.1 % level

Since the primary objective of the aerobic plate count is to evaluate the sanitational aspect of food handling, aerobic mesophilic count at 37°C would be deemed sufficient for this purpose. But such a count will not reflect the actual microbial load on the food where psychrotrophic organisms are also involved as in the case of raw fish or frozen products. The microbial flora of raw fish contained species of which a good part was classified as psychrotrophs. Our earlier studies had indicated that raw fish (*Sardinella longiceps*) contained about 44% of psychrotrophs while mesophiles constituted only 56%. It was also found from their growth temperature studies that 31% of the bacteria isolated from fresh fish could not grow at 37°C (Thampuran & Iyer, 1979). It was not surprising therefore that 30°C incubation is giving higher counts.

In frozen samples, the predominating flora is Gram positive cocci belonging mainly to genus *Micrococcus* (Thampuran & Iyer unpublished data). Selection of cold resistant species due to freezing and frozen storage coupled with the elimination of a large number of strict mesophilic types would have been the reason for getting higher counts at 30°C.

Generally it was found that spread plates yielded more colonies than pour plates for raw and frozen fish. Clark (1967) had suggested that spreading by means of a glass rod would allow the clumped cells to get disintegrated thereby improving the dispersion of cells resulting in higher counts. But analysis of our data shows that the count difference between spread plates and pour plates at 30°C is greater than the count difference at 37°C. Had the effect been due to clumping of the cells alone this difference would not have arisen. It appears therefore that higher counts obtained in spread plates could be due to other factors as well.

One possibility may be the sensitivity of certain marine micro-organisms to the action of hot agar used in pour plating. Zobell & Conn (1940) pointed out the harmful action of agar at 45°C on certain marine bacteria. The extreme heat sensitivity of a psychrotrophic bacterium *Pseudomonas fluorescens* was recently shown by Gray *et al.* (1973). Nottingham *et al.* (1975) suggested that the lower count in pour plates could be attributed to the inability of psychrotrophs to survive in the hot agar media used in pour plating.

Postgate (1967) stated that bacteria subjected to a stress becomes hypersensitive to a secondary stress. It has been shown that microbial starvation which can occur in low nutrient environments can also lead to increased susceptibility to a transient secondary warming stress (Klein & Shenyu Wu, 1974). This was shown by decreased recoveries of heterotrophic microorganisms from aquatic environments in pour plate as compared to spread plate method. In frozen foods, stress due to intense cold can cause the microbial flora to be more sensitive to warm agar temperature of 45°C leading to decreased recoveries with pour plate method.

The implication of this study was limited by the number of samples analysed and methods of enumeration adopted. The data nevertheless points out the significance of adopting a procedure by which greater accuracy is attained in the estimation of bacterial count of sea foods.

The authors are grateful to the late Shri. G. K. Kuriyan, former Director, Central Institute of Fisheries Technology for his keen interest and encouragement. They are also thankful to the technicians in the laboratory for specimen collection and laboratory assistance.

References

- AOAC (1970) *Official Methods of Analysis*, 11th edn. Association of Official Analytical Chemists, Washington
- Clark D. S. (1967) *Can. J. Microbiol.* **13**, 1409
- Gray, R. J. H., Witter, L. D. & Ordahl, Z. J. (1973) *Appl. Microbiol.* **26**, 78
- IS: 8653 (1977a) *Indian Standard Specification for Sardines, Fresh*. 1st edn. Indian Standards Institution, New Delhi
- IS: 8652 (1977b) *Indian Standard Specification for Sardine, Frozen*. 1st edn. Indian Standards Institution, New Delhi
- Jan Gjerde (1975) *Fisk Dir Skr.* **1**, 17
- Klein, D. A. and Shenyu Wu (1974) *J. Appl. Microbiol.* **27**, 429
- Lee, J. S. & Lee ann Harward (1970) *J. Milk Fd Technol.* **33**, 237
- Mossel, D. A. A. & Van der Moosdijk, A. (1963) *J. appl. Bact.* **26**, 10
- Nottingham, P. M., Rush brook, A. J. & Jury, K. E. (1975) *J. Fd Technol.* **10**, 273
- Postgate, J. R. (1967) *Advanced Microbial Phys.* **1**, 1
- Shenyu, Wu & Klein, D. A. (1976) *J. Appl. Environ. Microbiol.* **31**, 216
- Stapert, E. M., Sokolski, W. T. & Northam, J. I. (1962) *Can. J. Microbiol.* **8**, 809
- Thampuran Nirmala & Iyer, K. M. (1979) *Fish. Technol.* **16**, 15
- Van Soestbergen, A. A. & Lee, C. H. (1969) *Appl. Microbiol.* **18**, 1092
- Zobell, C. E. & Conn, J. E. (1940) *J. Bact.* **40**, 223

Seasonal Changes in the Biochemical Composition of Ovary in *Heteropneustes fossilis* (Bloch)

KALPANA D. SHRENI

Department of Zoology, Aligarh Muslim University, Aligarh-202 001

Seasonal changes in the biochemical composition of ovary in *H. fossilis* are reported. An inverse relationship was noted in fat and water contents. Maximum fat was observed in June and lowest in December. Protein and ash were generally low during winter and high during summer or monsoon months. Variations in the cholesterol content were more or less identical to those of the fat.

Ovary is the important organ controlling the metabolism in female fish. Chemical changes occur when fish becomes sexually mature. In female fish, body constituents are mobilized from different tissues to the ovary for the concoction of the eggs. Studies on its chemical composition and food value are of paramount importance. Observations on seasonal variations have been carried out by various workers (Bailey *et al.*, 1952; Idler & Bitners, 1958; Jafri, 1968 a, b; 1969; Jafri & Khawaja, 1968; Love, 1970; Mc Cartney, 1966; Shreni 1980, Shreni & Jafri, 1977; Siddiqi, 1966; Somvanshi, 1979). However, information on seasonal changes in various biochemical constituents of the ovary is scanty. Hence an attempt has been made to investigate variations in the biochemical composition of the ovary in cat fish, *H. fossilis* (Bloch) during different months.

Materials and Methods

Ovaries collected from fishes obtained from local market were weighed and dried on an oven at 100°C to constant weights. The experimental methods employed for the estimation of fat, water, protein, ash and cholesterol were the same as reported by Shreni (1980) and Shreni & Jafri (1977). All the determinations were made in triplicate.

Results and Discussion

Seasonal changes in the biochemical composition of ovary of female fish (Table 1) were significant and had some correlation to various activities of the female fish such as feeding and spawning.

Percentage of fat varied from 1.67 (in December) to 6.96 (in June), whereas the percentage of water fluctuated between 58.11 (in June) to 84.55 (in December). The values of fat were high during the prespawning and spawning months, thus progression in maturation was accompanied by a rapid accumulation of fat in the ovarian tissue and consequently, the highest values of fat were observed at peak ripening period. A distinct fall in the ovarian fat during September and a gradual rise in subsequent months were characteristic of the spent and recovering phases. The rise and fall in the ovarian fat were also accompanied with a rise and fall in the weight of the ovaries. A high degree of relationship between the gonadal fat and maturity has earlier been pointed out in several fishes (Jafri, 1968 a, b, 1969; Jafri & Khawaja, 1968). The fat and water showed an inverse relationship. Moisture values were high in the spent, immature and the maturing specimens. Low moisture values in ovary during ripe stage may be to accommodate more reserves.

The protein values varied from 8.49 (in January) to 27.49 (in August). A greater accumulation of protein in the ovary was found associated with maturation and ripeness. A gradual fall after August coincided with the spent stage. A rise in the total ovarian protein during maturation has also been observed in many other fishes (Jafri, 1968 a, b; 1969; Jafri & Khawaja, 1968).

The change in values of ash in ovary are given in Table 1. The ash percentage varied from 0.59 (in December) to 2.04

Table 1. Monthly mean values of fat, water, protein, ash and cholesterol in the ovary of *H. fossilis* (Bloch)

	Fat %	Moisture %	Protein %	Ash %	Cholesterol (mg/100 g wet tissue)
January	1.88	83.61	8.49	1.12	607.70
February	1.72	83.14	9.20	1.09	241.30
March	2.00	79.76	14.24	1.06	366.70
April	2.19	77.45	18.02	1.63	551.70
May	5.88	61.90	25.29	1.91	1031.30
June	6.96	58.11	24.42	2.00	1435.60
July	6.30	60.79	26.13	2.04	1579.80
August	5.43	63.18	27.49	1.76	1516.40
September	2.67	64.76	21.49	1.71	1153.42
October	2.95	71.29	14.75	1.78	1194.30
November	2.03	80.93	11.45	1.96	783.60
December	1.67	84.55	11.10	0.59	730.30

(in July). There was more rapid increase of ash in ovarian tissue consequent on maturation. Maximum ash values observed during May–July were found associated with ripening of ovary. A fall in the ash content similarly characterized the spawning and postspawning phases of gonad maturation. Higher ash values during maturation probably indicate an enhanced mineral metabolism of the fish.

The cholesterol values varied from 241.30 (in February) to 1579.80 (in July). There seemed to be little correlation between the ovarian cholesterol cycle and feeding. However, it was found to synchronize well with its cycle of maturation and depletion. Ripening was associated with a rapid synthesis and accumulation of cholesterol in the ovary and the highest values in July coincided with a peak ripeness. A fall was noticed with the onset of spawning in August which continued in subsequent months. During the recovering spent phase the ovary always contained less cholesterol. From these observations, it appears that advancement in maturation brings about a mobilization of cholesterol from reserves to ovary for its development and this may serve as the precursor of sex hormones (oestrogens) and of other steroid hormones.

A corollary to our findings is also apparent in the work of Channon & El-Saby (1932), who observed a steady loss of cholesterol from the liver and intestine and a rapid gain by the gonad in herring, but Idler & Bitners (1960) and Idler & Tsuyuki (1958) recorded a fall in the cholesterol content of serum, liver and gonad of both sexes of sockeye salmon, *Oncorhynchus nerka* during maturation. The observed changes in the cholesterol distributional pattern of ovary of this fish may thus be related to changes in the cholesterol metabolism encountered during the maturation of the fish and necessitated beside other factors, by the demand for sex hormones.

The author is grateful to Prof. N. H. Khan, Head of Zoology for facilities and to Dr. A. K. Jafri, Reader in Zoology, Aligarh Muslim University for valuable suggestions. Thanks are also due to CSIR for a fellowship, during the tenure of which this work has been carried out.

References

- Bailey, B. E., Carter, N. M. & Swain, L. A. (1952) *Bull. Fish. Res. Bd Can.* 89, 413
- Channon, H. J. & El-Saby, M. K. (1932) *Biochem. J.* 26, 2021

- Idler, D. R. & Bitners, I. (1958) *Can. J. Biochem. Physiol.* **36**, 793
- Idler, D. R. & Bitners, I. (1960) *J. Fish. Res. Bd Can.* **17**, 113
- Idler, D. R. & Tsuyuki, H. (1958) *Can. J. Biochem. Physiol.* **36**, 783
- Jafri, A. K. (1968 a) *Broteria*. **37**, 29
- Jafri, A. K. (1968 b) *Broteria*, **37**, 45
- Jafri, A. K. (1969) *Hydrobiologia*. **33**, 497
- Jafri, A. K. & Khawaja, D. K. (1968) *Hydrobiologia*. **32**, 206
- Love, R. M. (1970) in *The Chemical Biology of Fishes*. Academic Press, New York
- McCartney, T. H. (1966) *Fish. Res. Bull. N. Y.* **29**, 72
- Shreni, K. D. (1980) *Proc. Indian Acad. Sci.* **89**, 191
- Shreni, K. D. & Jafri, A. K. (1977) *Fish. Technol.* **14**, 116
- Siddiqi, M. A. (1966) *Indian J. Exp. Biol.* **4**, 122
- Somvanshi, V. S. (1979) *Fish. Technol.* **16**, 39

Microbiological Evaluation and Keeping Quality of Fishes Reared in Livestock Sewage Fed Ponds Without Artificial Feed

V. N. BACHHIL

L.P.T. Division, Indian Veterinary Research Institute, Izatnagar-243 122

Livestock sewage has been utilized for fish culture. There is lack of information on microbiological evaluation and keeping quality of these fishes. This paper reveals the incidence, types of micro-organisms and keeping quality of fishes reared in livestock sewage fed ponds without artificial feed. These fishes revealed microbial incidence and keeping quality comparable to other fishes. Initial mesophilic and psychrophilic counts varied from 3.38 to 5.56 and 2.47 to 4.74. On an average, the counts reduced by about 40% after evisceration and washing. Whole as well as washed fishes had refrigerated ($8 \pm 1^\circ\text{C}$) life of not more than 4 days. The average psychrophilic and mesophilic counts of ice (0 to 1°C) stored whole fishes upto 10th day varied from 3.66 to 4.81, 4.61 to 5.24 and in eviscerated and washed fishes 2.17 to 3.69 and 2.78 to 4.41. Both remained acceptable till the 10th day. Qualitative study of surface slime and gills revealed presence of *Aerobacter* (*Enterobacter*), *Aeromonas*, *Alcaligenes*, *Bacillus*, *Clostridia*, *E. coli*, *Klebsiella*, *Micrococci*, *Proteus* and *Pseudomonas*.

Exploitation of resources to meet the ever increasing protein gap, has given rise to the concept of waste utilization. Livestock sewage which usually goes as a waste, has been used in fish culture with a view to recycle the nutrient and water. Only little work has been done in the country using livestock sewage in fish culture and probably no data on microbiological evaluation of sewage reared fishes is therefore available. Contrary to this, exhaustive reviews on microbiological aspects are available for marine and other fresh water fishes. Both types of fishes are known to harbour various types of bacteria on the skin surface (Nair & Lahiry, 1968; Frazier, 1967; Jay, 1970 and Shewan, 1961).

Under present investigation, the fishes were reared in ponds fed with livestock sewage without artificial feed. This paper describes the incidence and type of micro-organisms normally associated with these fishes; the pattern of their mesophilic and psychrophilic growth during storage and subsequent spoilage along with their keeping quality during refrigerated ($8 \pm 1^\circ\text{C}$) and iced (0 to 1°C) storage.

Materials and Methods

Fishes were obtained from composite fish culture pond of the institute. They were brought to the point of sale from where rohu fishes of about 1.0 to 1.5 kg were collected for experiment. Half of the fishes were immediately eviscerated and washed with tap water and the rest were subjected to analysis as whole fishes. Whole as well as washed fishes were divided into two sets each. First set was stored at $8 \pm 1^\circ\text{C}$ in refrigerator and the second in ice at 0 to 1°C . Mesophilic and psychrophilic microbial enumeration along with the organoleptic examination of fishes in each set was carried out on 1st (0 to 1h), 4th, 7th and 10th days.

Measured area of skin surface (Jay, 1970) was swabbed using sterile template under aseptic precaution and the swab placed in 10 ml of 0.1% sterile peptone water (IS, 1976). It was agitated and mixed well to give 1:10 dilution. Further serial ten fold dilutions were prepared from this tube. Pour plates in quadruplicate were prepared from each of the three consecutive dilutions.

using standard plate count agar (IS, 1969). Two plates of each dilution from each set were incubated at 37°C for 24 ± 1 h to obtain total mesophilic count. Rest of the plates were incubated at 5°C for 5 to 7 days for psychrophilic count. Colonies were counted with the help of electronic colony counter and reported as count/cm² of the examined surface. Microbial load reported and discussed in this paper are in logarithmic scale. Generic identification of the bacteria isolated was done according to Jay (1970).

Results and Discussion

Range and average mesophilic and psychrophilic counts of whole as well as washed fishes are presented in the Table 1. Initial psychrophilic population of whole fishes was lesser than the mesophiles. Higher population of mesophiles can be attributed to warmer climate. In warmer waters, such as those of India, more mesophiles are encountered and the peak bacterial load in seas are also said to coincide with maximum water temperature (Georgala, 1958; Shewan, 1961). On an average, initially mesophiles and psychrophiles were observed to be 4.61 and 3.66. Shewan (1961), Nair & Lahiry (1968) mentioned heavy microbial load in marine fishes in the range of 2 to 7 and 2 to 5/cm². Nair *et al.* (1971) reported total viable count of 4.139/cm² from fresh water fishes. The microbial load of fishes reared under present system was in agreement with the general pattern mentioned above. Initial counts were observed to be much below the higher limit occurring in marine fishes (Shewan, 1961). Microbial counts increased during refrigeration at $8 \pm 1^{\circ}\text{C}$ (Table 1). On 4th day, both the types of fishes were organoleptically acceptable without any visible sign of spoilage. On 7th day, whole fishes exhibited signs of spoilage with mean mesophilic and psychrophilic population of 6.86 and 5.98 respectively. Eviscerated and washed fishes with mean mesophilic and psychrophilic count of 6.27 and 4.8 also emitted off odour, sliminess, sunken eyes and textural changes. As refrigerated fishes were organoleptically fresh on 4th day but exhibited deteriorative changes on 7th day ie after six days of storage, the onset of spoilage can be presumed to have occurred

in 4 to 5 days indicating its refrigerated life of not more than 5 days.

There was reduction in mesophilic and psychrophilic populations by about 40% after evisceration and washing. Washed fishes for a while kept better (Frazier, 1967); it could be due to low initial count, but afterwards population build up and more exposure of opened surface to external contamination, hastens the process of deterioration in quality of eviscerated and washed fishes.

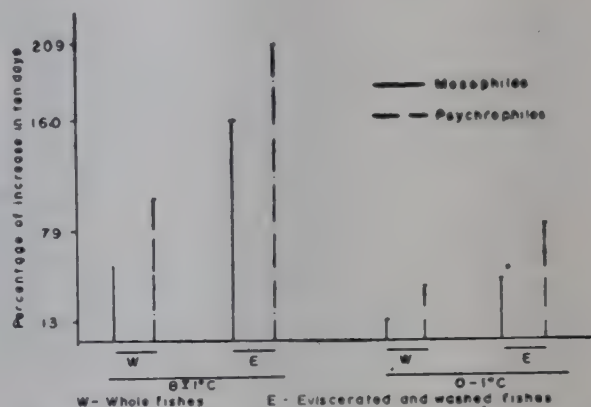


Fig. 1. Per cent increase over initial microbial load of fishes reared in livestock sewage fed ponds

Fig. 1. shows that the increase in microbial population of ice stored fishes was much less than those stored at $8 \pm 1^{\circ}\text{C}$. Nair & Lahiry (1968) cited various workers who showed main spoilage bacteria of fishes as psychrophilic. Figure 1 shows the per cent increase to be more in psychrophilic group as compared to mesophiles. Jay (1970) described the spoilage organisms to grow in surface slime. This explains the increase in surface microbial load in present study.

Icing still remains to be the widely used method of fish preservation. Table 1 shows that the range of counts both in mesophilic and psychrophilic group were irregular during iced storage. Nair *et al.* (1971) reported the irregular growth pattern on skin due to washing action of ice particularly during re-icing. The psychrophilic and mesophilic count in whole fishes were around 4-5/cm² throughout the iced storage. It was still less in eviscerated and washed fishes. Nair *et al.* (1971) reported

Table 1. Influence of storage temperature and period on microbial load and keeping quality of fishes reared in livestock sewage fed ponds

Temperature	Storage		Whole fishes		Eviscerated and washed fishes	
	Days	No. of fishes	Mesophiles	Psychrophiles	Mesophiles	Psychrophiles
$8 \pm 1^\circ\text{C}$	1	10	R 3.38–5.56	2.47–4.74	1.79–4.88	1.11–3.96
			M 4.61	3.66	2.78	2.17
	4	5	R 4.99–6.93	4.50–5.55	2.57–5.79	2.00–4.79
			M 5.73	4.83	4.34	3.20
	7	5	R 5.82–7.82	4.94–6.88	4.93–7.43	2.13–6.81
			M 6.86	5.98	6.27	4.80
	10	5	R 7.00–8.25	7.00–8.80	6.00–8.2	5.94–8.34
			M 7.75	7.44	7.20	6.72
0 to 1°C	1	10	R 3.38–5.56	2.47–4.74	1.79–4.88	1.11–3.96
			M 4.61	3.66	2.78	2.17
	4	5	R 2.08–6.91	3.0–5.84	2.96–4.75	2.51–4.00
			M 5.09	4.17	3.80	3.05
	7	5	R 4.24–6.30	2.68–5.50	3.71–4.83	2.00–4.43
			M 5.24	4.43	4.41	3.60
	10	5	R 2.88–7.39	2.59–6.00	2.81–5.04	2.91–4.27
			M 5.23	4.81	3.97	3.69

R = Range of counts; M = Mean microbial load expressed in logs cm^2

viable bacterial count of fresh water fishes reaching 6 to 7 after 5 weeks storage whereas in ice stored marine fishes total viable count of this magnitude reaches in 9 to 10 days. They further reported the bacterial load of ice stored fresh water fishes to be $5.81/\text{cm}^2$ on 12th day. This approximates to our observations. Fishes reared in livestock sewage thus have comparable keeping quality to other fresh water fishes and that the count does not reach to the level of marine fishes after 9 days of iced storage.

Ice stored fishes in both the groups did not reveal any sign of spoilage. There was no off odour but the eyes were sunken particularly in the whole fishes. Eviscerated and washed fishes looked bright and acceptable. Keeping in view the sunken eyes and texture of muscle in whole fishes, the eviscerated fishes were better in appearance and acceptability. Banik *et al.* (1976) made almost similar observation when they reported storage life of eviscerated and washed fishes in ice as 12 days. They reported microbial counts of 4.43 and 5.49 for white pomphret and Indian mackerel

after 7 days. Mean microbial counts were still less under present study and these remained acceptable upto 10th day. Nair *et al.* (1974) while studying chilled storage of fresh water fishes also reported the samples to be organoleptically good at the end of 10 days with bacterial population in the range of 5.41 to 5.67/g in rohu.

Surface slime and the gills examined for naturally occurring microflora of these fishes revealed presence of *Aerobacter* (Enterobacter), *Aeromonas*, *Alcaligenes*, *Bacillus*, *Clostridia*, *E. coli*, *Klebsiella*, *Micrococci*, *Proteus*, and *Pseudomonas*. Frazier (1967) and Jay (1970) also described presence of all these bacteria in marine as well as in fresh water fishes except *Aerobacter* and *Klebsiella*. Surface slime and gills have been reported to harbour various bacteria (Shewan, 1961; Jay, 1970). The presence of *Aerobacter* and *Klebsiella* is logical due to the fact that pond water is contaminated with sewage and surrounding vegetation. According to WHO (1974), fishes reared in sewage contaminated water carry organisms of faecal origin,

namely, *Klebsiella*, *E. coli* and *Clostridia*. When fishes are close to land, organisms of terrestrial origin also occur and in such cases *Bacillus* is in abundance (WHO, 1974).

Thanks are due to Director, I. V. R. I., for providing the facilities.

References

- Banik, A. K., Chaudhary, D. R. & Bose, A. N. (1976) *J. Fd Sci. Technol.* **13**, 67
- Frazier, W. C. (1967) *Food Microbiology*. Mc Graw Hill Book Co. New York
- Georgala, D. L. (1958) *J. Gen. Microbiol.* **18**, 84
- IS 5402 (1969) *Indian Standards—Food Microbiology*, Indian Standards Institution, New Delhi
- IS 5887 (1976) part-1 *Indian Standards—Food Microbiology*, Indian Standards Institution, New Delhi
- Jay, J. M. (1970) *Modern Food Microbiology*, p. 76, Van Nostrand & Reinhold Co. New York
- Nair, R. B. & Lahiry, N. L. (1968) *J. Fd Sci. Technol.* **5**, 107
- Nair, R. B., Tharamani, P. K. & Lahiry, N. L. (1971) *J. Fd Sci. Technol.* **8**, 53
- Nair, R. B., Tharamani, P. K. & Lahiry, N. L. (1974) *J. Fd Sci. Technol.* **2**, 118
- Shewan, J. M. (1961) in *Fish as Food*. **1**, p. 487, Academic Press, New York
- WHO (1974) *Rep. WHO Expt. commi. Tech. Rep. Ser. 550* World Health Organisation, Geneva

Survey on the Idle Capacity of Fish Processing (Freezing) Plants in India—I. West Coast

H. KRISHNA IYER, P. SRINIVASA RAO, G. R. UNNITHAN, A. K. KESAVAN NAIR
and R. G. NAIR

Central Institute of Fisheries Technology, Cochin-682 029

The extent of idle capacity in fish processing (freezing) plants estimated by stratified random sampling is reported. The estimates for 1978 and 1979 for the processing plants on the west coast of India were 76.9% and 73.2% respectively at the rate of 250 working days per annum and two shifts per day. The percentage error of estimates worked out to 6.04 for 1978 and 6.98 for 1979. Substantial under utilization of processing plants noticed in all the states accounts mainly to the non-availability of raw material (prawn), high cost of production and shortage of power.

Fish processing industry in India has registered remarkable growth during the last decade. Indian marine product export touched a record figure of 92.2 thousand tonnes during 1979 (Anon, 1979). The foreign exchange earning from marine products showed an impressive eight fold increase during 1969 to 1979, the figures being Rs. 330.7 million for 1969 and Rs. 2620.3 million for 1979 (Anon, 1979). The share of frozen prawns, lobster tails and froglegs contributed 87% in 1969 and 95% in 1979. The flourishing trend in the fish processing industry, over the years, attracted many new entrepreneurs to this field and consequently a number of prawn freezing plants with varying capacities sprang up along the coast. With the increase in fish processing factories, the total installed capacity also had increased considerably, and there were reasons to suspect large scale unutilized capacity in these plants at present due to the seasonality of the raw material. Since, large idle capacity of plants is not at all desirable for the economic functioning of any industry, especially the fish processing industry, the authors felt the need for conducting an all India survey in 1980 to estimate the extent of idle capacity in fish processing plants in India during the years 1978 and 1979, to identify the causes responsible for it and to suggest ways and means to reduce the same for the economic functioning of the plants. The findings of the survey for the different maritime states in the west coast of India are reported in this paper.

Materials and Methods

There were 275 fish processing (freezing) plants in India in 1979, processing frozen prawns, froglegs and lobster tails and occasionally squid and cuttle fish. Out of these, 181 were in the west and 94 were in the east coasts. They were stratified according to the installed capacity of 5 tonnes and below, 5 to 10 tonnes and above 10 tonnes per day. Fixing the sampling error at 20% on the total installed capacity, a sample of 93 plants were selected for the study. The technique adopted was that of stratified random sampling (Sukhatme & Sukhatme, 1970).

Data on installed capacity of the plants, actual production during the year, factors responsible for the under utilization of the plants, number of personnel employed, sources of raw material and ice and cold storage facilities available in the plants were collected from the sampled factories through personal interviews with the plant managers in each state for 1978 and 1979. The total idle capacity for each stratum was estimated using the formula,

$$\hat{Y}_h = \frac{N_h}{n_h} \sum_{i=1}^{n_h} Y_{hi}$$

where N_h is the total number of plants in the h^{th} stratum, n_h is the number of plants sampled from the h^{th} stratum and Y_{hi} is

the idle capacity of the i^{th} plant included in the sample from the h^{th} stratum. The strata estimates were pooled at the respective levels so as to get the estimates of idle capacity for each state, for east and west coasts and for India as a whole. For example, the total idle capacity for west coast as a whole was estimated as

$$\sum \hat{Y}_h$$

the summation running for all the strata in the west coast. Variance of the estimated total idle capacity of each stratum was estimated by

$$V(\hat{Y}_h) = \frac{N_h(N_h - n_h)}{n_h} \times \frac{1}{n_h - 1} \left(\sum_{i=1}^{n_h} Y_{hi}^2 - \frac{\left(\sum_{i=1}^{n_h} Y_{hi} \right)^2}{n_h} \right)$$

The estimated variances separately for the states on the west and east coasts and the country as a whole were obtained by pooling the estimated strata variance at the respective levels.

The idle capacity of each sampled plant was worked out by taking the difference between the installed capacity and actual production during the year for single, double and triple shifts on a normal working day. For purposes of annual capacity estimates, a day with two shifts is considered as the normal working day with respect to many organised industries. In fish processing industry too, one with two shifts per day can be considered a normal working day. But this can be true only for plants having their own fishing boats. As all the plants do not possess their own boats, the authors have worked out the annual capacity on a single, double and triple shift basis for comparison. The number of normal working days in a year were taken to be 200 and 250, though under practical conditions, 250 days are normal with respect to many organised industries (Mensinkai, 1969). The remaining 100 days were sufficient to cover off season, work stoppages, holidays and repairs of the plants. However, considering the availability of raw material for processing and the processing practices existed in different states, the estimates of idle capacity were worked out by taking 200 and 250 normal working days in a year.

The percentage error of estimates for the states, west and east coasts and India as a whole were worked out using the formula,

$$\frac{\sqrt{\sum V(\hat{Y}_h)}}{\sum \hat{Y}_h} \times 100$$

the summation running over all the strata at the respective levels. The percentage idle capacity was worked out by taking the ratio of the underutilised capacity to the installed capacity for single, double and triple shifts.

Results and Discussion

The estimates of installed capacity, idle capacity and the percentage error of estimates worked out for the different states on the west coast are presented in Table 1, for all the three shifts and for 200 and 250 working days in a year for 1978 and 1979. The total installed capacity of all the plants with single, double and triple shifts for 250 working days were 110.4, 220.7 and 331.1 thousand tonnes in 1979 while the estimated total production during the year was 59.3 thousand tonnes (Table 1). The percentage idle capacity for the three shifts (250 days) were 54, 77 and 85 during 1978 as against 46, 73 and 82 during 1979. The percentage error of estimates of idle capacity in 1978 and 1979 for double shift with 250 working days were 6.04 and 6.98 respectively, indicating the reliability of the estimates. Table 2 gives the percentage idle capacity in different stratum for the west coast states in 1978 and 1979.

Kerala

Kerala, on the west coast has the maximum number of freezing factories and is the pioneer state to begin export of frozen prawns. The industry flourished here for quite a number of years. Kerala with a coast line of 560 km had 104 (57% of the total fish processing factories on the west coast) factories in 1979. Of these, 69 were

below 5 tonnes, 19 were between 5 to 10 tonnes and 16 over 10 tonnes of daily capacity. 16 plants were sampled for the study. The total installed capacity of all the plants in the state estimated were 56.1, 112.1 and 168.2 thousand tonnes respectively for single, double and triple shift for 250 working days in a year (Table 1). The total estimated production for all the plants was 27.5 thousand tonnes in 1978 and 29.1 thousand tonnes in 1979. The estimates of unutilised capacity of the plants in double shifts were 84 (for 1978) and 83 thousand tonnes (for 1979) for 250 working days. The percentage idle capacity worked out for the three shifts with 250 working days were 48.0, 74.0 and 82.7 respectively in 1979. It could be seen from Table 1 that only 26% of the installed capacity was utilised in this state during 1979 (250 working days with 2 shifts per day). The brake up figures in different strata (Table 2) showed that idle capacity was comparatively low in plants of 5 to 10 tonnes capacity for both the years. A majority of the plants in Kerala (66%) were under 5 tonnes, mostly of 2 to 2.5 tonnes per day. This small sector had to face strong competition in the procurement of raw material (prawn). The unsteady foreign markets and unsound financial position made them unable to compete with bigger entrepreneurs resulting in substantial under utilisation of plants under 5 tonnes capacity. Bigger plants (above 10 tonnes) were unable to procure sufficient raw material owing to its scarcity. The major factors responsible for the idle capacity of plants in Kerala as per the survey were nonavailability of raw material (prawn), high cost of production, labour problems and frequent power failures. The percentage error of estimates for double shifts with 250 working days in 1978 and 1979 were 8.70 and 8.65 respectively, indicating the reliability of the estimates.

Karnataka

Karnataka has a coast line of 270 km and there were 29 fish processing plants in 1979 (16% of the total on the west coast). Of these, 18 are below 5 tonnes, 9 are 5 to 10 tonnes and 2 are above 10 tonnes capacity. 10 plants were sampled for the study. The estimated annual installed capacity of these plants (Table 1) during 1979 for single, double and triple shift were 11.2

22.4 and 33.6 thousand tonnes for 200 working days and the corresponding figures for 250 working days were 14.0, 28.0 and 42.0 thousand tonnes. The estimated production during 1979 was 5.7 thousand tonnes. The estimates of idle capacity for the three shifts in 1978 were 9.3, 23.9 and 37.3 thousand tonnes and the corresponding figures in 1979 were 8.3, 22.3 and 36.3 thousand tonnes based on 250 working days. Compared to 1978 there was slight decrease of idle capacity in 1979. On the basis of double shift and 250 working days in a year, only 20% of the installed capacity was utilised in 1979. The percentage idle capacity in different strata (Table 2) showed that idle capacity was comparatively less in plants under 5 tonnes capacity for all the three shifts of 200 and 250 working days. The causes for under utilisation of the plants in this state as revealed by the survey were nonavailability of raw material and the high cost of production. The percentage error of estimates of idle capacity for 250 working days with double shift in 1978 and 1979 were 8.61 and 7.88 respectively.

Goa

There were 8 fish processing plants functioning along the 110 km coast line of Goa in 1979. 6 were under 5 tonnes and 2 were 5 to 10 tonnes capacity and 5 were sampled for the study. The estimated annual installed capacities for single, double and triple shift with 250 working days in 1979 were 3.7, 7.3 and 11.0 thousand tonnes while the total production was 2.7 thousand tonnes. The percentage idle capacities estimated for the 3 shifts were respectively 27.2, 63.6 and 75.7 (Table 1). Compared to other states on the west coast, idle capacity was less in Goa, because a part of the raw material for the plants were supported by the catches of Zuary and Mandhovi rivers. Among the plants in different strata (Table 2), plants below 5 tonnes had comparatively less idle capacity, 49.7% in 1978 and 50.2% in 1979 for double shift with 250 working days. Non availability of raw material and high cost of production were the major factors contributing to the idle capacity of plants in this region. The percentage error of estimates in 1978 and 1979 for double shift with 250 working days were

Table 1. Annual installed capacity and the rate of unutilised capacity (in thousand tonnes) in 1978 and 1979 for different states

	Single shift				Double shift				Triple shift			
	200 days		250 days		200 days		250 days		200 days		250 days	
	1978	1979	1978	1979	1978	1979	1978	1979	1978	1979	1978	1979
Kerala												
a) Annual installed capacity	44.9	44.9	56.1	56.1	89.8	89.8	112.1	112.1	134.7	134.7	168.2	168.2
b) Estimated idle capacity	17.4	15.8	28.6	26.9	62.3	60.7	84.0	83.0	107.2	105.6	139.4	139.1
c) % idle capacity	38.8	35.1	50.9	48.0	69.4	67.6	74.9	74.0	79.6	78.4	82.9	82.7
d) % error of estimates	34.5	36.2	21.6	21.6	10.9	10.9	8.7	8.7	7.3	7.3	6.3	6.3
Karnataka												
a) Annual installed capacity	11.2	11.2	14.0	14.0	22.4	22.4	28.0	28.0	33.6	33.6	42.0	42.0
b) Estimated idle capacity	6.5	5.5	9.3	8.3	17.7	16.7	23.9	22.3	28.9	27.9	37.3	36.3
c) % idle capacity	58.4	49.2	66.8	59.3	79.2	74.6	85.3	79.7	86.1	83.1	88.9	86.4
d) % error of estimates	22.6	27.2	16.6	18.5	10.2	10.8	8.6	8.9	7.7	7.9	6.9	7.0
Goa												
a) Annual installed capacity	2.9	2.9	3.7	3.7	5.9	5.9	7.3	7.3	8.8	8.8	11.0	11.0
b) Estimated idle capacity	1.5	0.3	2.5	1.0	4.4	3.2	5.9	4.7	7.3	6.1	9.5	8.3
c) % idle capacity	49.7	9.0	67.7	27.2	74.9	54.5	79.9	63.6	83.3	69.6	86.5	75.7
d) % error of estimates	17.7	*	10.0	22.2	4.8	6.3	3.2	3.6	2.3	3.3	1.4	2.0
Maharashtra												
a) Annual installed capacity	22.1	22.1	27.8	27.8	44.3	44.3	55.5	55.5	66.6	66.6	83.2	83.2
b) Estimated idle capacity	7.9	4.4	14.5	10.1	29.9	26.6	41.0	37.8	52.0	48.9	68.5	65.6
c) % idle capacity	35.6	20.0	52.1	36.4	67.6	60.1	73.8	68.2	78.1	73.5	82.4	78.8
d) % error of estimates	40.8	*	24.4	52.9	18.5	25.4	16.8	20.9	15.9	18.6	15.1	16.8
Gujarat												
a) Annual installed capacity	7.1	7.1	8.9	8.9	14.2	14.2	17.8	17.8	21.3	21.3	26.6	26.6
b) Estimated idle capacity	3.9	3.0	5.7	4.8	11.0	10.1	14.5	13.6	18.1	17.2	23.4	22.5
c) % idle capacity	54.8	42.1	63.8	53.7	77.4	71.1	81.9	76.9	84.9	80.7	87.9	84.6
d) % error of estimates	—	—	—	—	—	—	—	—	—	—	—	—
West coast as a whole												
a) Annual installed capacity	88.2	88.2	110.4	110.4	176.4	176.4	220.7	220.7	264.6	264.6	331.1	331.1
b) Estimated idle capacity	37.1	28.9	59.3	51.1	117.1	117.1	169.7	161.4	213.5	205.3	280.0	271.8
c) % idle capacity	42.1	32.8	53.7	46.3	71.1	66.4	76.9	73.2	80.7	77.6	84.6	82.1
d) % error of estimates	18.8	27.0	12.3	17.9	7.1	7.7	6.0	7.0	5.4	5.6	4.9	5.3

* In some of the freezing plants, the actual production exceeded the installed capacity for single shift.

Table 2. Statewise percentage idle capacity in different strata for 1978 and 1979

	Shifts	1978						1979					
		200 days			250 days			200 days			250 days		
		U/5	5/10	10 up	U/5	5/10	10 up	U/5	5/10	10 up	U/5	5/10	10 up
Kerala	1	38.7	Nil	75.6	50.9	9.5	80.4	21.6	1.2	76.0	37.0	20.9	80.8
	2	69.4	43.1	87.8	74.1	54.7	90.2	60.8	50.7	88.0	68.6	60.4	90.4
	3	79.6	62.4	91.9	81.8	69.8	93.5	73.9	67.1	92.0	79.1	73.6	93.6
Karnataka	1	37.6	69.6	91.2	50.2	75.7	92.9	40.8	42.6	87.6	52.7	54.1	90.1
	2	68.8	84.8	95.7	79.2	87.9	96.5	70.4	71.3	93.8	76.3	77.0	95.1
	3	79.2	89.9	97.1	83.4	91.9	97.7	80.3	80.9	95.9	84.2	84.7	96.7
Goa	1	24.3	73.0	—	32.8	78.4	—	Nil	67.4	—	0.5	73.9	—
	2	45.5	86.5	—	49.7	89.2	—	37.8	83.7	—	50.2	87.0	—
	3	52.5	91.0	—	55.4	92.8	—	58.5	89.1	—	66.0	91.3	—
Maharashtra	1	46.0	24.8	37.4	56.8	56.9	49.5	47.3	7.9	18.6	57.9	26.3	35.5
	2	72.9	62.4	68.1	78.5	69.9	74.3	73.7	53.9	59.7	78.9	63.1	67.8
	3	81.9	75.0	78.4	85.7	79.9	82.6	82.4	69.3	73.1	85.9	75.4	78.5
Gujarat	1	—	70.6	50.4	—	76.5	60.3	—	44.2	41.5	—	55.4	53.2
	2	—	85.3	75.2	—	88.3	80.2	—	72.1	70.8	—	73.7	76.6
	3	—	90.2	83.5	—	92.2	86.8	—	81.4	80.5	—	85.1	84.4
West coast as a whole	1	39.1	29.4	57.9	51.2	36.4	66.4	24.7	16.3	49.4	39.7	33.1	59.6
	2	69.6	60.2	78.9	75.6	68.2	83.2	62.3	58.2	74.7	69.8	66.5	79.8
	3	79.7	73.5	86.0	83.7	78.8	88.8	74.8	72.1	83.1	79.9	77.7	86.6

respectively 3.23 and 3.63 indicating the reliability of the estimates.

Maharashtra

Maharashtra has a coast line of 600 km and there were 32 fish processing plants in the state during 1979. Out of these, 11 were under 5 tonnes, 10 were 5 to 10 tonnes and 11 were above 10 tonnes capacity and 9 were sampled for the study. The estimated installed capacity of all the plants for single, double and triple shifts during 1979 for 250 working days were 27.8, 55.5 and 83.2 thousand tonnes (Table 1) respectively while the production estimate for the year was 17.7 thousand tonnes. The estimates of idle capacity for the 3 shifts in 1979 were respectively 10.1, 37.8 and 65.6 thousand tonnes and the percentage idle capacity were 36.4, 68.2 and 78.8 respectively. Compared to 1978 (Table 1) there was an improvement in capacity utilisation in 1979 due to improved prawn landing in this state. Next to Goa, Maharashtra showed less percentage idle capacity in all the 3 shifts. Among different strata (Table 2), plants of 5 to 10 and above 10 tonnes showed less percentage idle capacity. The major factors contributing to the idle capacity of plants in this state were also nonavailability of raw material, high cost of production and power shortage. The percentage of estimates for 1978 and 1979 for double shift with 250 working days were 16.79 and 20.85 respectively.

Gujarat

Gujarat with maximum coast line of 1500 km had 8 fish processing plants in 1979 and all were surveyed for the study. Of the 8, three were 5 to 10 and 5 above 10 tonnes capacity. The installed capacity of all the plants during 1979 for the 3 shifts with 250 working days were 8.9, 17.8 and 26.6 thousand tonnes (Table 1) respectively while the production during the year was 4.1 thousand tonnes. The percentage idle capacity of the plants for the 3 shifts (250 days) were respectively, 53.7, 76.9 and 84.6 in 1979. Compared to 1978, there was a slight improvement in capacity utilisation in 1979, due to improved prawn catch during the year. The stratumwise figures (Table 2) of percentage idle capacity

showed that plants of above 10 tonnes had less idle capacity in all the shifts with 200 and 250 working days. The reasons for underutilization of plants in this state were nonavailability of raw material, shortage of power and shortage of potable water.

Table 3. *Factors responsible for under-utilisation of plants*

Factors	% of plants reported in the sample
Nonavailability of raw material	89.6
High cost of production	52.1
Frequent power failures/shortage	29.1
Labour troubles	16.7
Unsteady foreign markets	10.4
Shortage of potable water	10.4
Cut throat competition for procuring the raw material	8.3
Shortage of ice	8.3
Lack of transport facilities	8.3
Lack of cold storage facilities	6.3
Investment in holding the material upto shipment	2.1
Delay in getting the purchase order.	2.1

Thus it is evident that there existed considerable extent of idle capacity of fish processing plants in the west coast of India and it was 76.9% in 1978 and 73.2% in 1979 for double shift with 250 working days (Table 1). The slight improvement in the utilized capacity in 1979 was mainly due to the freezing of squids, cuttle fish and fresh fish by a few plants in this coast.

A list of factors responsible for the large idle capacity of the plants in the west coast were presented in Table 3. It is evident that nonavailability of raw material was the main contributing factor for the substantial underutilization of plants. High cost of production, shortage of power and frequent power failures were other major

factors. Based on the answers to the questionnaire furnished by the processors, the following are a few recommendations which may help to reduce the idle capacity of the fish processing plants in the west coast.

1. Promoting mass aqua culture of prawn to meet the raw material scarcity.
2. Diversification of products.
3. Subsidy to diesel oil for fishing boats.
4. Improvement in shipping facilities.
5. Liberalisation of bank loans for small processors.
6. A check on issuing licence to new entrepreneurs.
7. Abolition of purchase tax on raw material
8. Mesh regulation in trawl nets.

The authors wish to express their deep sense of gratitude to all the fish processors in India for extending their wholehearted co-operation in supplying the required data for the study. They are also thankful to Dr. C. C. Panduranga Rao, Director, Central Institute of Fisheries Technology and Dr. P. N. Kaul, Scientist S-3 for encouragements.

References

- Anon (1979) *Statistics of Marine Products Exports*. The Marine Products Export Development Authority, Cochin
- Mensinkai, S. S. (1969) *Plant Location and Plant Size in the Fish Processing Industry of Newfoundland*. *Can. Fish. Rep.* **11**, 18
- Sukhatme, P. V. & Sukhatme, B. V. (1970) *Sampling Theory of Surveys with Applications*, 2nd edn., Asia Publishing House, New Delhi.

Studies on Blue Discolouration in Canned Body Meat of Crab (*Scylla serrata*)

P. K. VIJAYAN and K. K. BALACHANDRAN

Central Institute of Fisheries Technology, Cochin-682 029

Blue discolouration met with in the canned body meat of crab (*Scylla serrata*) was due to copper content exceeding 1.8 to 2.0 mg/100g on dry weight basis. Bleeding the cleaned carcasses of crab thoroughly in running water brought down the copper content below this level and blue discolouration prevented. Addition of copper ions to the thoroughly bled crab meat to raise the copper content above this level caused discolouration. The incidence of blue discolouration was independent of the freshness of the animals used. Citric acid in high concentration prevented blueing to some extent, but is not advisable as texture and flavour were adversely affected.

Blue discolouration in canned crab meat is a common phenomenon which has engaged the attention of several workers in identifying the causative factors and its prevention. Most of the literature on the subject suggest that copper ion is mainly responsible for its occurrence. Goringer & Dassow (1964) observed that the blued product in King crab (*Paralithodes camtschatica*) had some properties similar to those of copper proteins or biuret complexes. Osaka (1958) succeeded in preventing the blue discolouration in canned crab meat by a "low temperature and fractional heating" of the carcasses from which carapaces were removed. Blackwood *et al.* (1959) recommended a fractional cooking method as a means of preventing the blue discolouration in canned Queen crab (*Chionoecetes opilio*) meat. Inoue & Motihiro (1970, a, b, c, d, e; 1971, 1971a) studied in detail the cause and mechanism of the blue discolouration in canned King crab meat and established a relationship between the copper content in the meat and incidence of the blue discolouration. However, Waters (1971), based on his studies on canned crab (*Callinectes sapidus*) inferred that only ferrous and ferric ions produced blue discolouration typical of that met with in canned crab meat. In his studies neither cuprous nor ammonium ions was found to cause significant blueing.

Crab meat forms a sizeable proportion of the canned marine products exported

from India. Its share in the total exports of canned products is presented in Table 1.

Table 1. *Export of canned marine products from India**

Item		1979	1978	1977
Shrimp	Q	139	204	128
	V	64.28	91.49	52.21
Crab meat	Q	56	42	50
	V	29.35	19.41	31.44
Tuna	Q	NEG	14	22
	V	0.15	2.20	3.49
Sardine	Q	—	NEG	NEG
	V	—	NEG	NEG
Fish	Q	2	1	—
	V	0.80	0.20	—
Mussel	Q	2	NEG	—
	V	0.48	0.13	—
Total	Q	199	261	211
	V	95.06	113.43	88.55

*Sources: "Indian Seafood Exports Scale New Heights, Rs. 262 Crores." Marine Products Export Development Authority, Cochin

Q: Qty. in tonnes; V: Value Rs. in lakhs

Sporadic occurrence of blue discolouration has been met with in the Indian canned crab meat when the body meat is canned separately or body and claw meats are canned together, the degree of incidence being higher in the latter case. Practically

no research work has been carried out in India on this problem. Attempts to analyse the cause and possible methods of prevention of this phenomenon in canned body meat of *Scylla serrata* is discussed in this paper.

Materials and Methods

Crabs of average length 15 cm caught from Cochin backwaters were processed after about 3 h. The carapaces and claws were removed and the resultant carcasses were halved and kept in running water for varying periods whenever required. The carcasses were cooked in 2% boiling brine until the meat became firm enough to facilitate easy removal by scissors, mixed thoroughly and packed in lacquered cans (301 x 109) with parchment lining on all sides, filled with hot brine (2% brine containing 0.1% citric acid) and heat processed at 110° C for 60 min.

Moisture was estimated by the method of A.O.A.C. (1975). The crab meat after drying was ashed, and the ash dissolved in a small volume of hydrochloric acid to give a 0.1 N solution on dilution with distilled water. The analysis for copper was done directly using a Varian Techtron atomic absorption spectrophotometer Model 1100. (Bryan, G.W.). Visual observations were made on the colour of the canned meat and gradings given according to the intensity of colour.

Results and Discussion

Freshly caught crab was divided into three lots. One processed immediately and the second and third were processed after exposing to the atmosphere for 4 and 8 h respectively. The first group of crabs processed were activity live, the second sluggish and the third near dead when processed. The canned meat from these three lots were examined for any incidence of blue discolouration. The results are presented in Table 2.

It was observed in several cases that meat picked from fresh crabs had the colour superior to those from sluggish or dead crabs. It is evident from the results that the blue colour is independent of the freshness of the crab used for processing.

Waters (1971) has reported that dead or decayed crabs do not cause any blue discolouration in canned meat. It has been observed in the present study that blueing whenever occurred, was irrespective of the freshness of the meat.

In another set of experiments, meat picked from fresh boiled crabs was packed in 2% brine containing varying concentrations of citric acid to study the effect, if any, of citric acid on prevention of blueing. The results are summarised in Table 3.

In one set of experiments the colour of samples turned blue when citric acid in brine was 0.3% or less and at 0.4% this phenomenon was apparently controlled.

Varga *et al.* (1969) carried out studies on the use of citric acid in preventing blueing in Queen crab (*Chionoecetes opilio*) meat. They observed that 0.1% citric acid considerably prevented the colour change and completely prevented the phenomena at higher concentration of the acid. Dipping the meat in acetic or citric acid solutions has been recommended for preventing blueing in European edible crab (*Cancer pagurus*) (Edwards & Early). However, this does not appear to be a recommendable process as the flavour and texture of the resultant product was very much adversely affected.

Copper has been reported to be a major factor responsible for the blue discolouration in canned crab meat. The copper content in a number of samples of raw crab meat was estimated and reported in Table 4, which shows that the copper content in crab meat varied between 0.09 and 5.696 mg/100g. In order to discern the relationship between the copper content in the meat and its influence on the blue discolouration of canned meat, meat showing blue discolouration in cans was separated out and the copper content estimated and compared with the copper content in normal meat. Table 5 shows a definite relationship between copper content and the occurrence of blue discolouration. Canned meat with very light blueing had a copper content of 1.81mg/100g and the intensity of the colour increased with increase in copper.

Inoue & Motihiro (1970) have established a relationship between the copper content

Table 2. *Effect of time lapse between catching and processing on the incidence of blue discolouration in canned body meat*

Experiment No.	State of crab used	Colour of meat	Visual rating of blueing
1	L	White	0
	S	White/ dull white	0
	D	Dull white	0
2	L	White	0
	S	White	0
	D	Dull white	0
3	L	White	0
	S	White/ dull white	0
	D	Dull white	0
4	L	White with slight blueing in parts	+
	S	White with slight blueing in parts	+
	D	Dull white with slight blueing in parts	+
5	L	White with moderate blueing in parts	++
	S	White with moderate blueing in parts	+
	D	Dull white with moderate blueing in parts	++

L = actively live, processed without delay

S = sluggish, processed after 4 h

D = dead/near dead, processed after 8 h

0 = no blueing

+ = slight blueing

++ = moderate blueing

Table 3. *Effect of citric acid in the prevention of blue discolouration*

Experiment No.	Concentration of citric acid in brine %	Visual rating of blueing
1	0 - Control	0
	0.1	0
	0.2	0
	0.3	0
	0.4	0
	0.5	0
	0 - Control	+
2	0.1	+
	0.2	+
	0.3	+
	0.4	0
	0.5	0
	0 - Control	+
	0.1	+

0 = normal; — no blueing; + = slight blueing; ++ = moderate blueing

Table 4. *Copper content in raw crab body meat*

Sample	Copper content (DWB) mg/100g
1	2.190
2	2.890
3	5.696
4	5.589
5	5.023
6	3.724
7	3.524
8	5.213
9	0.090
10	0.160

Table 5. *Correlation between copper content and blue colour in canned crab meat*

Sample	Copper content (DWB) mg/100g	Visual rating of blueing
1	0.06	0
2	0.095	0
3	1.62	0
4	1.82	— +
5	2.043	+
6	2.89	++
7	3.34	+++
8	4.33	+++

0 = no blueing; — + = very slight blueing; + = slight blueing; ++ = moderate blueing; +++ = heavy blueing

and the incidence of blue discolouration in the meat of King crab. They found 0.49mg/100g copper in normal King crab meat and 2.8mg/100g in the blue crab meat and stated that the blue colour appears when copper exceeds 22mg/100g. Their studies on the cause and mechanism of blue discolouration in canned crab (Inoue & Motihiro, 1970a, 1971, 1971a) showed 1970b, that it is due to the haemocyanin contained in crab haemolymph, which can react with hydrogen sulphide to produce blue colour. They observed that the reflectance spectra of haemocyanin-sulphide complex closely resembled that of the blue meat and concluded that the causative factor for blue

discolouration in canned crab is a haemocyanin-sulphide complex.

Since the blood haemocyanin of crab is the source of copper in the meat, different methods were tried to bleed the animals to get rid of copper. In order to increase the exposed area to facilitate bleeding the cleaned carcasses were halved and then subjected to treatments as detailed in Table 6.

The data indicates that it is advantageous to bleed the carcasses in running water for 30 min. To find out the optimum time required for bleeding and to bring down the copper content to a satisfactory level, the cleaned carcasses were bled in running water for varying periods and the copper contents estimated after bleeding and the meat canned and observed for the incidence of blueing. The results are presented in Table 7.

When the initial copper content was low, bleeding for 15 min in running water was sufficient to bring down the copper content to a satisfactory level when no blue colour developed in the canned meat. When the initial copper content was very high, bleeding upto 45 min did not yield satisfactory results. Longer bleeding in such cases, not only failed to prevent the appearance of blue colour but also affected the texture and flavour of the meat. However, cases wherein such abnormally high copper content is met with are very rare and for practical purposes bleeding for 30 min in water was quite sufficient to prevent the incidence of blue colour in the product.

The "low temperature and fractional heating" method described by Osakabe employs the principle of bleeding of the crab carcasses to get rid of the copper making use of the difference in the coagulating temperatures of blood and muscle proteins. Similar method has been recommended by Blackwood *et al.* (1969) for prevention of blueing. However bleeding the cleaned carcasses of crab in water was found to be simple and sufficient from this study.

Tables 5 and 7 indicate that the blue discolouration becomes apparent when the copper in the meat is around 2 mg/100g. Known amounts of copper in the form of

Table 6. *Copper content in the body meat of raw crab after various treatments*

Treatment	Copper content mg/100g (DWB)	
	Experiment 1	Experiment 2
Raw body meat (control)	2.89	3.524
Bled in running water for 30 min	1.80	1.910
Dipped in 2% brine for 30 min	2.31	2.620
Dipped in 2% brine containing 0.1% citric acid for 30 min	2.29	2.710

Table 7. *Effect of duration of bleeding on the copper content and the incidence of blueing*

Copper content mg/100g (DWB) and visual rating of blueing

Time of bleeding min	I		II		III	
	Copper	Visual rating	Copper	Visual rating	Copper	Visual rating
10	2.19	0	2.89	+	5.199	+++
15	1.829	0	2.17	0	3.720	++
30	1.62	0	1.81	0	2.920	+
45	1.50	0	1.72	0	2.160	0

0 = no blueing; + = slight blueing; ++ = moderate blueing; +++ = heavy blueing

Table 8. *Effect of added copper ions on blueing*

Copper (as CuCl ₂) added mg	Copper in canned meat mg/100g (DWB)	Visual rating of blueing
Control	1.628	0
0.5	1.916	— +
1	2.54	++
1.5	4.36	+++

0 = no blueing; — + = very slight blueing; ++ = moderate blueing; +++ = heavy blueing

cupric chloride solution were added to the meat picked from thoroughly bled and cooked crab carcasses to find out the effect of added copper ions in the occurrence of blue discolouration and also to determine the critical level of copper needed for the appearance of blue colour. It is observed

from Table 8 that by introducing copper ions blue colour occurred in the sample with a copper content of 1.916mg/100g. This is almost in agreement with the observations recorded in Tables 5 and 8.

From the experiments and observations made above it can be reasonably concluded that the blue discolouration in canned crabs is due to copper content around 2 mg/100g and by bringing down the copper content below this level by bleeding the meat in water, the phenomenon of blue discolouration can be prevented in canned crab meat.

References

- A.O.A.C. (1975) *Official Method of Analysis*. Association of Official Analytical Chemists, 12th edn., Washington
- Blackwood, C. M., Varga, S. & Dewar, A. B. (1969) Meeting on Atlantic Crab Fishery Development, Fredericton-N. B. March 4-5

- Bryan, G. W. (1973) *J. mar. biol. Ass. U. K.* **53**, 145
- Edwards, E. & Early, J. C. *Torry Advisory Note No. 26; Torry Research Station, Aberdeen*
- Goringer, H. S. & Dassow, J. A. (1964) *Fish Ind. Res.* **2**, 47
- Inoue Norio & Motihiro, T. (1970) *Bull. Jap. Soc. Sci. Fish.* **36**, 588
- Inoue Norio & Motihiro T. (1970a) *Bull. Jap. Soc. Sci. Fish.* **36**, 692
- Inoue Norio & Motihiro, T. (1970b) *Bull. Jap. Soc. Sci. Fish.* **36**, 695
- Inoue Norio & Motihiro, T. (1970c) *Bull. Jap. Soc. Sci. Fish.* **36**, 945
- Inoue Norio & Motihiro, T. (1970d) *Bull. Jap. Soc. Sci. Fish.* **36**, 1040
- Inoue Norio & Motihiro, T. (1970e) *Bull. Jap. Soc. Sci. Fish.* **36**, 1044
- Inoue Norio & Motihiro, T. (1971) *Bull. Jap. Soc. Sci. Fish.* **37**, 1007
- Inoue Norio & Motihiro, T. (1971a) *Bull. Jap. Soc. Sci. Fish.* **37**, 1011
- Osakabe (1958) *Marine Products in Japan*, (Tanikawa, E. Ed.), 179, Koseisha Koseikaku Company, Japan
- Varga, S., Dewar, A. B. & Anderson, W. E. (1969) *Meeting on Atlantic Crab Fishery Development*, Fredericton, N. B., March 4-5
- Waters, M. E. (1971) *Spl. Sci. Rep.* **633**, U. S. Department of Commerce, N.O.A.A., N.M.F.S., Seattle, Washington

Influence of Sex, Spawning, Starvation and Water Temperature on Fatty Acid Composition in *Tilapia mossambica*

K. G. RAMACHANDRAN NAIR and K. GOPAKUMAR

Central Institute of Fisheries Technology, Cochin-682 029

The influence of sex, spawning, starvation and water temperature on the fatty acid composition of *Tilapia mossambica* has been studied. Tilapia egg lipid was found to have unusually high percentage of C_{22:6} fatty acids (9.09%) compared to body and intestinal lipids. The C_{16:1} acid was much less in the egg lipids (3.5%) whereas it was 11% in the body lipids. There was no significant difference in the fatty acid composition of body and intestinal lipids of male and female tilapia. Starvation caused the presence of high content of lower fatty acids (C₆, C₈, C₁₀, C₁₂ and C₁₄) in the body lipids. Water temperature also influenced the fatty acid composition of *Tilapia*; the difference was more significant in body lipids than in intestinal lipids.

The influence of dietary fatty acids on the fatty acid composition of body and intestinal lipids of fish has been reported by Kaneko *et al.* (1967 a), Kelly *et al.* (1958), Braekkan *et al.* (1971) and Nair & Gopakumar (1981). The effects of seasonal changes, sex, water temperature and starvation on the fatty acid composition of fish were also reported by a number of workers (Rieser *et al.* 1963; Kaneko *et al.* 1967 b; Ackman & Eaton 1971, and Inui & Ohshima 1966). The influence of sex, spawning, starvation and water temperature on the fatty acid composition of body and intestinal lipids of *Tilapia mossambica* is reported in this paper.

Materials and Methods

Fully grown male and female fishes were allowed to breed in the laboratory. The juveniles were subsequently separated and kept in separate tanks. They were provided with a formulated diet, the fatty acid composition of which was reported by Nair & Gopakumar (1981).

To study the role of sex and effect of spawning in the fatty acid composition, lipid was extracted separately from the body and intestine of six months old ones following the method of Bligh & Dyer (1959). Lipids were extracted from females before and after spawning and from egg also. The

fatty acid composition is presented in Table 1.

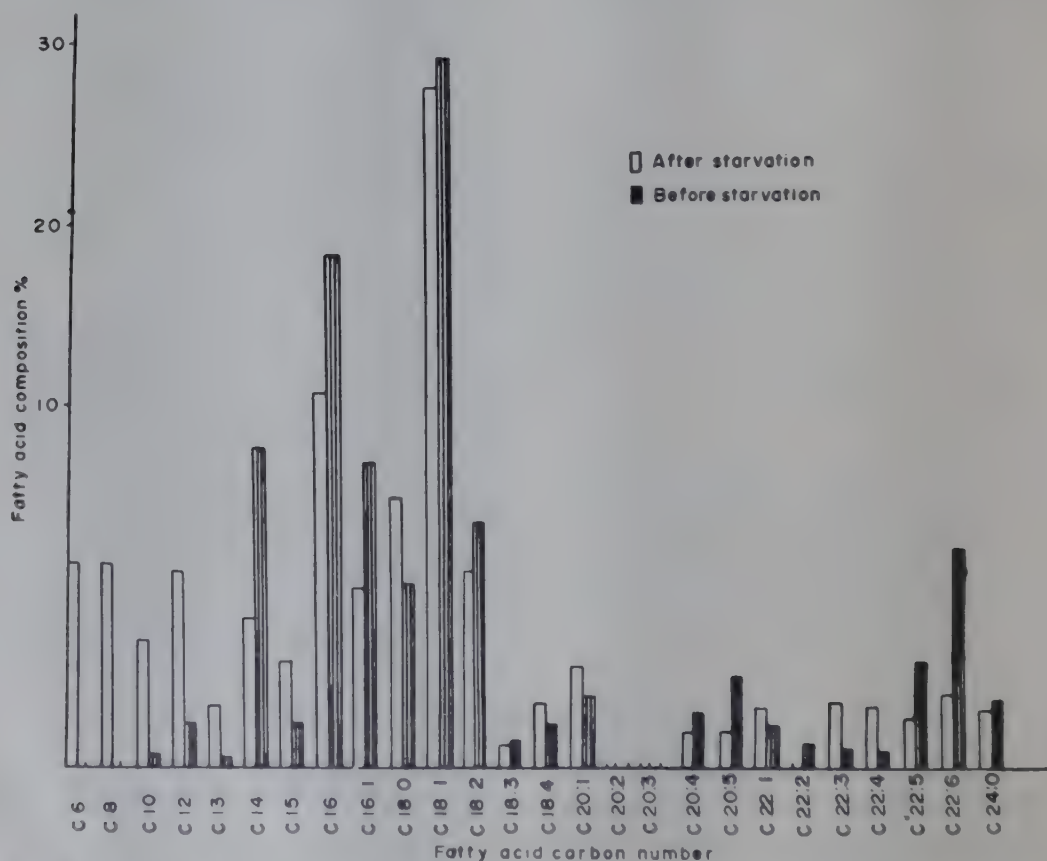
To determine the effect of starvation, forty fishes were kept in another tank and allowed to starve for 30 days under aeration. After 30 days the fishes became very sluggish. They were killed and lipid extracted from the body and intestine separately. The proximate composition before and after starvation (Table 2) was determined by the method of AOAC (1975). The fatty acid composition is given in Figs. 1 and 2.

For understanding the effect of temperature on fatty acid composition, fishes grown in the tank at 27±1°C was taken as the ambient temperature. Twenty fishes were kept in a tank at 17±1°C and another 20 at 37±1°C. Temperature was maintained by an electronic immersion thermostat and heater. The water was constantly aerated and fishes were fed with the same diet. After one month, lipid was extracted from the body and intestine of fishes kept at three different temperatures. Fatty acid composition is given in Figs. 3 and 4.

From the lipid samples, fatty acid methyl esters were prepared using BF₃ methanol reagent as per AOAC (1975) and stored in ampoules, sealed under nitrogen and kept in deep freezer.

Table 1. Effect of sex and spawning on fatty acid composition of *Tilapia*

	Female (Before spawning)			Female (After spawning)		Male	
	Body	Intestine	Egg	Body	Intestine	Body	Intestine
C ₁₂	1.50	1.70	0.46	0.7	0.6	1.99	3.86
C ₁₃	—	—	—	0.14	0.20	—	—
C ₁₄	5.23	5.79	4.00	4.09	6.00	5.01	7.47
C ₁₅	2.14	1.37	1.63	1.65	2.00	1.63	1.00
C _{16:0}	26.07	24.24	25.38	24.52	27.00	20.25	19.29
C _{16:1}	10.86	11.11	3.52	14.66	13.80	12.65	12.29
C _{18:0}	7.85	8.62	10.47	8.24	9.70	8.5	6.97
C _{18:1}	28.41	25.41	24.34	24.05	17.70	25.86	29.12
C _{18:2}	6.61	6.91	8.20	6.52	7.75	8.40	8.36
C _{18:3}	2.80	4.1	3.78	3.3	3.89	3.79	3.24
C _{18:4}	0.47	1.38	1.02	1.29	1.21	1.76	1.23
C _{20:1}	—	0.8	—	0.33	0.36	—	—
C _{20:2}	0.19	0.59	—	0.35	0.43	0.52	0.78
C _{20:3}	0.31	0.53	1.06	0.53	0.43	1.10	0.6
C _{20:4}	1.79	1.92	3.3	2.00	2.01	2.00	1.34
C _{20:5}	0.37	0.59	0.53	0.84	0.66	0.66	0.45
C _{22:2}	—	—	0.32	0.33	0.25	0.25	—
C _{22:3}	0.78	0.59	0.73	0.35	0.40	0.50	0.3
C _{22:4}	0.52	0.46	0.95	0.33	0.36	0.49	0.2
C _{22:5}	1.12	1.34	1.66	1.50	1.75	1.21	0.97
C _{22:6}	2.87	3.34	9.09	3.91	3.50	3.45	2.13

Fig. 1. Effect of starvation on the fatty acid composition of body fat of *Tilapia mossambica*

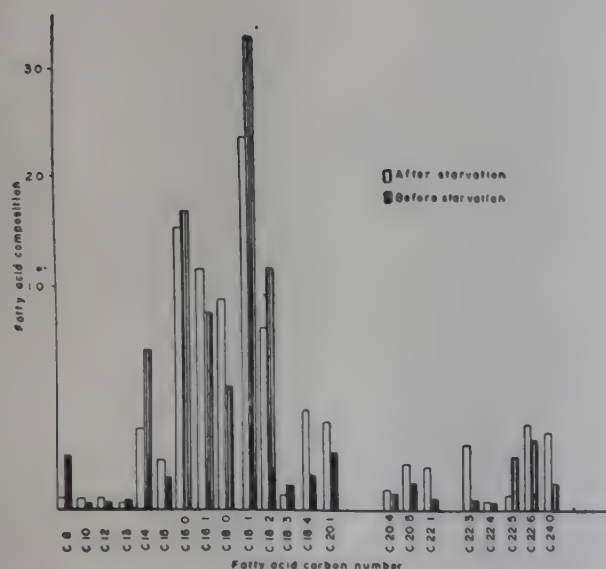


Fig. 2. Effect of starvation on the fatty acid composition of intestines with contents of *Tilapia mossambica*

Table 2. Proximate composition of *Tilapia* muscle before and after starvation

	Before starvation	After starvation
	%	%
Moisture	69	74.5
Ash	2.7	2.75
Protein	13.3	13.4
Fat	11.5	2.3

The fatty acid methyl esters were analysed in a gas chromatograph equipment (Toshniwal India Ltd.) using flame ionisation detector and strip chart recorder (10 mv, Varian Techtron; chart speed 30 cm/h). The column was made of stainless steel (182.88 cm x 6.35 mm o.d.) packed with silar 10C, 10% on Anakrom ABS (110-120 mesh). Operating conditions were as follows. Column temperature 196°C, injection port temperature 250°C, detector 275°C, carrier

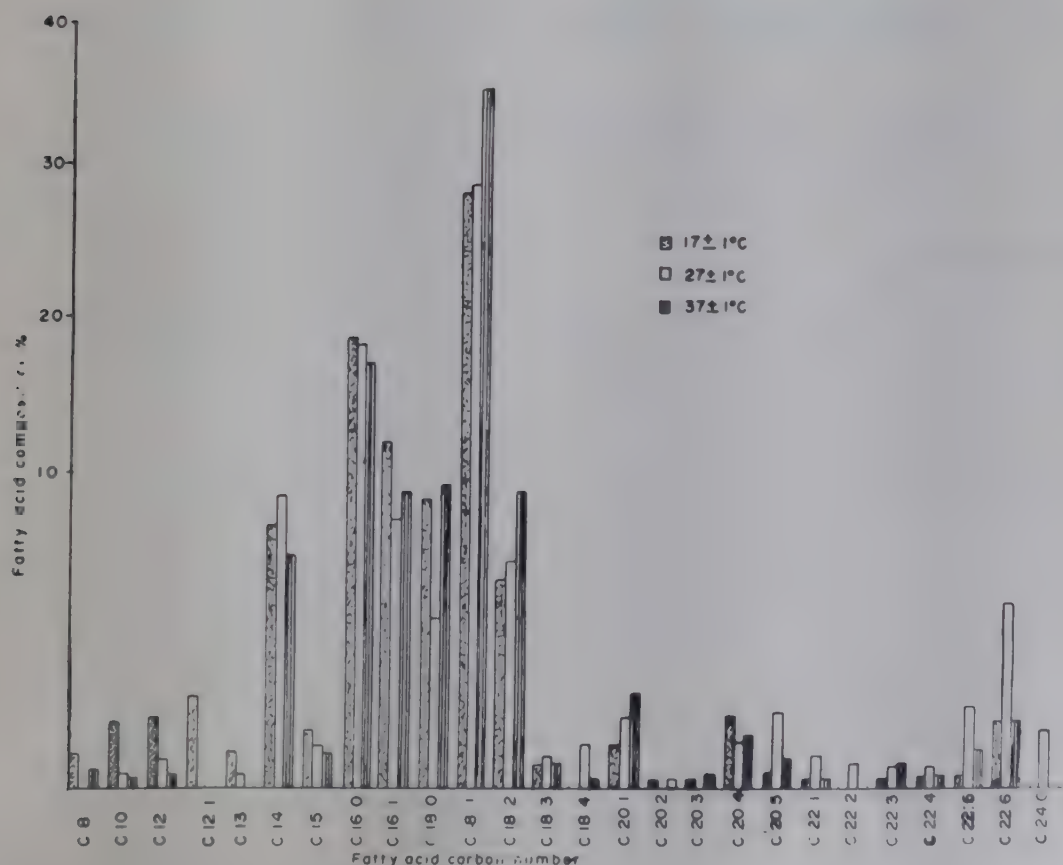


Fig. 3. Effect of temperature on fatty acid composition of body fat of *Tilapia mossambica*

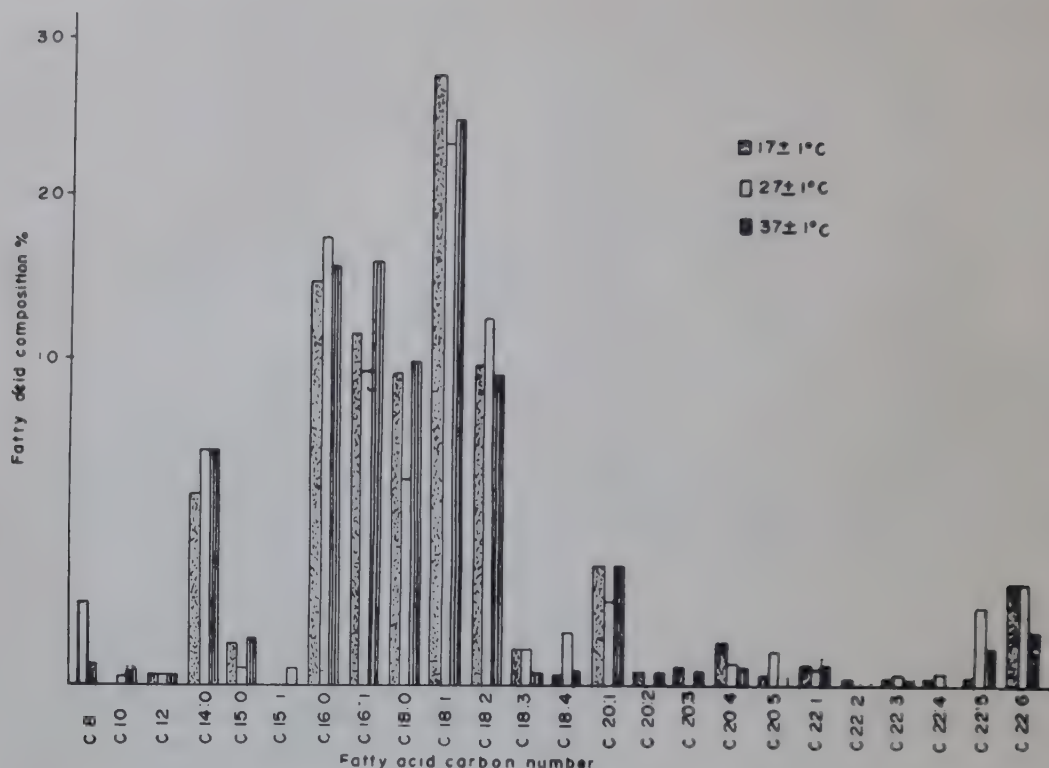


Fig. 4. Effect of temperature on the fatty acid composition of intestines with contents of *Tilapia mossambica*

gas nitrogen 40 ml/m. Identification and quantification were done as reported earlier (Ramachandran & Gopakumar, 1977).

Results and Discussion

Effect of spawning on the fatty acid composition of *Tilapia* was in agreement with the observation reported by Ackman *et al.* (1963). *Tilapia* egg lipids were found to have unusually high content of C_{22:6} acid (9.10%), whereas the body and intestines before and after spawning contained only about 3.5%. C_{20:4} acid was also slightly higher in the case of *Tilapia* egg lipids. C_{18:1} acid was very low (3.5%) compared to body lipids (11%). There is no significant variation in fatty acid composition either in body or intestines for both male and female similar to the observation of Ackman & Eaton (1971).

Effect of starvation

After one month's starvation, the body lipid was reduced to 2.3% from 11.5% and the intestinal lipids to 4.4% from 30.7%.

Starvation caused significant loss in weight also. Average weight of a fish decreased from 23.5 to 17 g. After starvation the body lipids were found to have unusually high content of C₈ (5.6%), C₈ (5.5%), C₁₀ (3.5%), C₁₂ (5.5%). C₁₃ (1.5%) C₆ and C₈ were absent in the case of *Tilapia* body fat before starvation. C₁₀, C₁₂ and C₁₃ were 0.4%, 1.2% and 0.3% respectively in the cases of body fat before starvation. After starvation concentration of C₁₄, C_{16:0}, C_{18:2}, C_{20:4}, C_{20:5}, C_{22:5} and C_{22:6} acids were considerably decreased. Since fat is the obvious source of energy for the fish under the period of starvation these acids would have undergone direct catabolism.

In the case of intestinal lipids, even though the total lipid content was subsequently decreased after starvation, no significant variation was observed in the fatty acids composition. After starvation fatty acid of following chain length C_{14:0}, C_{16:0}, C_{18:1}, C_{18:2} and C_{18:3} acids were found to be reduced in the intestinal lipids. But after starvation C_{16:1}, C_{18:4}, C_{20:1}, C_{20:4}, C_{20:5}, C_{22:1}, C_{22:3}, C_{22:6}, C_{24:0},

are more in the intestinal lipids compared to body lipids.

Effect of temperature

The fatty acid composition of *Tilapia* grown at different temperatures showed significant variations especially in body fat. Fatty acid C_8 , C_{10} and C_{12} were found to be slightly high in body fat of fish grown at $17\pm 1^\circ\text{C}$, whereas fish at $27\pm 1^\circ\text{C}$ and $37\pm 1^\circ\text{C}$ showed very little of these acids. At $17\pm 1^\circ\text{C}$, the body was found to contain $C_{12:1}$, 2.7%, whereas this was absent in the fish at $27\pm 1^\circ\text{C}$ and $37\pm 1^\circ\text{C}$. At temperature $17\pm 1^\circ\text{C}$ body fat contained 1.2% $C_{13:0}$ at $27\pm 1^\circ\text{C}$ it was only 0.3% and absent at $37\pm 1^\circ\text{C}$. At $37\pm 1^\circ\text{C}$, $C_{18:1}$ was 34.5%, at $27\pm 1^\circ\text{C}$, 27.9% and at $17\pm 1^\circ\text{C}$ it was 28.5%. The levels of $C_{22:6}$ acid was 6% at $27\pm 1^\circ\text{C}$ and 2.2% at $17\pm 1^\circ\text{C}$ and $37\pm 1^\circ\text{C}$. $C_{20:4}$ was more than $C_{20:6}$ at $17\pm 1^\circ\text{C}$, and $37\pm 1^\circ\text{C}$ but it was in the reverse order at $27\pm 1^\circ\text{C}$.

The effect of temperature on quantitative variation was not so significant in the case of other fatty acids.

Intestinal lipids also showed alteration in fatty acid composition with variation in temperature. At temperature $27\pm 1^\circ\text{C}$, $C_{16:0}$ was 17.1%, but it was only 14.5% and 15.0% at $17\pm 1^\circ\text{C}$ and $37\pm 1^\circ\text{C}$ respectively. But this trend was reversed for $C_{18:1}$ acid. At $27\pm 1^\circ\text{C}$ it recorded the lowest level (8.9%). Similar trend was observed in the case of $C_{18:0}$ (at $27\pm 1^\circ\text{C}$, 5.3%, $17\pm 1^\circ\text{C}$, 8.78% and at $37\pm 1^\circ\text{C}$ 9.4%). Marginal variations were also seen for $C_{18:3}$ and $C_{18:4}$ with alteration in adaptation temperature.

On the whole it can be seen that spawning, starvation and variation in adaptation temperature have got significant influence on the fatty acid composition in *Tilapia*.

References

- Ackman, R. G., Jangaard, P. M., Burgher, R. D., Hughes, M. L. & MacCallum, W. A. (1963) *J. Fish. Res. Bd Can.* **20**, 591
- Ackman, R. G. & Eaton, C. A. (1971) *Can. Inst. Fd Technol. J.* **4**, 169.
- AOAC (1975) *Official Methods of Analysis*. Association of Official Analytical Chemists, Washington
- Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911
- Braekkan, D. R., Lambertsen, G. & Andersen, J. (1971) *Report of Technological Research Concerning Norwegian Fish Industry, Directorate of Fisheries, Norway*, **5**, 5
- Inui, Y. & Ohshima, Y. (1966) *Bull. Jap. Soc. Sci. Fish.* **32**, 492
- Kelly, P. B., Rieser, R. & Hood, D. W. (1958) *J. Am. Oil Chem. Soc.* **35**, 503
- Kaneko, T., Takeuchi, M., Ishii, S., Higashi, H. & Kikuchi, T. (1967 a) *Bull. Jap. Soc. Sci. Fish.* **33**, 47
- Kaneko, T., Takeuchi, M., Ishii, S., Higashi, H. & Kikuchi, T. (1967 b) *Bull. Jap. Soc. Sci. Fish.* **33**, 56
- Nair, K. G. R., & Gopakumar, K. (1971) *J. Fd Sci. Technol.* **15**, 268
- Nair, K. G. R. & Gopakumar, K. (1981) *J. Fd Sci. Technol.* **18**, 108
- Rieser, R., Stervenson, B., Kayama, M., Choudhury, R. & Hood, D. W. (1963) *J. Am. Oil Chem. Soc.* **40**, 507

Comparative Study of the Nutrient Content of Fish and Shell Fish*

M. K. MUKUNDAN, A. G. RADHAKRISHNAN, M. A. JAMES and M. R. NAIR
Central Institute of Fisheries Technology, Cochin-682 029

The amino acid, mineral and proximate composition of mullet (*Mugil oeur*), mackerel (*Rastrelliger kanagurta*), crab (*Scylla serrata*) and prawn (*Penaeus indicus*) are reported. The data are used for comparing the nutritional quality of the fish and shell fish. Further, the amino acid composition is screened for their adequacy to meet the FAO/WHO recommended pattern of essential amino acids.

Fish is assuming greater importance in human diet owing to its superior nutritional quality and easy digestibility. It is necessary to know which of the fishes are nutritionally superior. The consumer is left with no idea other than the age old conventions to guide him in the selection of nutritious fish due to lack of sufficient data on this aspect of fish.

Studies on the biochemical composition and nutritive value of fish are few. Richard *et al.* (1962) and Sohn *et al.* (1961) reported the proximate composition of commercially important fishes of New England. Kutty Ayyappan *et al.* (1976) and Gopalan *et al.* (1980) studied the proximate composition of some Indian fishes. Mukundan & James (1977) and Mukundan *et al.* (1979) have worked out the nutrient distribution in a few tropical fishes. There are also reports on the distribution of specific nutrients such as sodium and potassium (Thurston & Claude, 1958), free amino acid composition (James, 1969 & Rangaswamy *et al.* 1970), methionine (Gowri *et al.* 1972) and glycine (Nair & Bose, 1965). The present paper reports the nutrient distribution in two fishes and two shell fishes and compares the proximate composition, mineral composition and amino acid make up between them.

Materials and Methods

Fresh adult fish and shell fish were used for the study. Mulletts were obtained from the catch of Chinese dipnets and mackerel from purse seine catches. Crab and prawns were collected from the backwaters of Cochin. The fish/shell fish were dressed and the edible portions separated and minced, immediately after death. For crab, both the body and claw meats were used. The minced samples were used for all the experiments. Prawns were peeled and deveined prior to mincing.

Moisture and ash were determined according to AOAC (1970) and fat by the method of Bligh & Dyer (1959). The ash was dissolved in 1 N hydrochloric acid for the determination of sodium, potassium and calcium (Vogel, 1960) and iron (APHA, 1976). Protein was estimated in 100 mg dry muscle after digestion with con. sulphuric acid as per Micro Kjeldhal method (Hawk, 1954).

Glycogen was extracted from the wet tissue according to Umbriet *et al.* (1959) and hydrolysed with 1 N hydrochloric acid, neutralised and colour developed with 0.2% Anthrone reagent in 95% con. sulphuric acid. The green colour developed from glucose was compared with standard glucose at 660 nm. Inorganic phosphorus was estimated in TCA extracts by the method of Fiske & Subbarow (1925). Amino acid composition was determined by standard microbiological assay (Kavanagh, 1963). All colorimetric measurements were done

*Paper presented in the seminar on 'Recent Trends in Teaching and Research in Aquatic Biology' organised jointly by Bhawanagar University, Central Salt and Marine Chemicals Research Institute, Department of Science and Technology, University Grants Commission, Indian Council of Agricultural Research and Government of Gujarat from September 26-28, 1980 at Bhavanagar.

in Spectronic 20 (Bosch and Lomb) and flame photometric measurements in a Systronic flame photometer.

Results and Discussion

The results of analyses of the major constituents are shown in Table 1 along with the computed calorific value. A close analysis of the data shows a clear distinction between fish and shell fish, the shell fish being relatively lean. This difference is well reflected in the higher caloric content of fishes. However this higher caloric content of fish is a highly variable factor owing to the seasonal changes in fat content of fish (Gopakumar, 1973). The moisture content of shell fish is comparatively higher than that of fish probably in accordance with the well known fat moisture relationship. Crab has a higher carbohydrate content. But this cannot be taken as a general feature of shell fish, as prawn records a carbohydrate value less than that of mullet. An overall view of the proximate composition shows that crab is characterised by high carbohydrate, moisture and low protein and fat in comparison with the other three which are more or less similar in their proximate composition.

Table 2 lists the mineral composition of fish and shell fish. Ash is significantly high in the muscle of mullet. The mineral composition showed no significant difference between fish and shell fish. Here also crab shows higher values for iron and calcium. However in calcium to iron ratio there is an important difference between fish and shell fish. Calcium and iron being indices of muscular activity (Smellie, 1974) and oxygen reception (White *et al.* 1973) respectively, their ratio can be considered to represent muscular activity per unit of oxygen consumed—'muscle index.' The muscle index is less for shell fish compared to fish, showing the possible superior muscular efficiency of fish. In nature, this condition is very much essential for fish, which lives by constant swimming from birth till death, while the shell fish mostly spend its time lying on the bottom floor.

Table 3 gives the amino acid composition of the four fishes studied and Table 4 presents the FAO/WHO (1973) recommended requirements of essential amino acids. As reported in some other fishes (Mukundan & James, 1977) all the fish and shell fish have a balanced distribution of all essential amino acids and 100 g protein from any of

Table 1. Proximate composition

Name of fish	Moisture g/100 g	Protein g/100 g	Fat g/100 g	Ash g/100 g	Glycogen g/100 g	Calorific value K. cal/100g
Mullet	75.77	20.22	2.45	1.62	0.90	105.53
Mackerel	71.19	21.21	7.51	1.33	0.50	154.40
Crab	79.23	17.50	0.21	1.39	2.70	82.69
Prawn	77.39	20.90	0.35	1.40	0.80	89.90

Table 2. Mineral composition

Name of fish	Sodium mg/100g	Potassium mg/100g	Calcium mg/100g	Inorganic phosphorus mg/100g	Iron mg/100g	Calcium/ iron
Mullet	99.08	411.3	357	185	4.3	83
Mackerel	100.16	424.5	429	308	4.6	93
Crab	186.80	378.8	680	150	10.2	67.8
Prawn	209.00	382.2	323	268	5.3	60.9

Table 3. *Amino acid composition (g/100 g protein)*

Amino Acid	Mullet	Mackerel	Crab	Prawn
Isoleucine	4.55	4.38	5.08	4.77
Leucine	5.8	4.97	6.49	8.34
Lysine	10.1	10.99	6.81	9.49
Methionine	2.33	3.46	4.81	4.29
Cystine	1.4	0.98	1.23	1.78
Phenyl alanine	4.25	3.3	4.53	6.63
Tyrosine	4.53	3.62	4.89	4.13
Threonine	4.16	4.32	5.7	4.64
Valine	6.51	4.53	4.53	4.53
Histidine	2.13	5.04	3.36	3.25
Glutamic acid	20.6	19.65	13.5	14.01
Tryptophan	0.69	1.24	1.02	0.98
Arginine	5.1	5.39	4.78	7.49
Serine	4.09	3.61	5.84	6.25
Proline	7.53	3.64	6.95	13.73
Aspartic acid	3.85	3.77	5.09	6.01
Glycine	4.18	2.47	4.63	6.18
Total essential amino acids	46.45	47.73	48.45	52.81
Total sulphur amino acids	3.73	4.34	6.04	6.07
Total aromatic amino acids	9.47	8.16	10.44	11.74

Table 4. *FAO/WHO recommended pattern of essential amino acid requirement per day (grams)*

Amino acid	Infant	Child	Adult
Isoleucine	3.5	3.7	1.8
Leucine	8.0	5.6	2.5
Lysine	5.2	7.5	2.2
Methionine + Cystine	2.9	3.4	2.4
Phenyl alanine	6.3	3.4	2.5
Threonine	4.4	4.4	1.3
Valine	4.7	4.1	1.8
Histidine	1.4	—	—

these fish/shell fish can provide more than double the amount of amino acids required for an adult per day. However the lysine requirement for child is limiting in these fish/shell fish except that in crab. Similarly the amino acid leucine is limiting in mullet, mackerel and crabs so far as the requirements of infants are concerned. Still, when plant and other animal proteins are considered, fish/shell fish are better sources of amino acids, especially in essential ones (Healdn, 1976).

Among fish and shell fish, there is a gradation in lysine content, the distribution of which is higher in fish. But the indices of total essential amino acids, sulphur amino acids and aromatic amino acids, which are nutritionally important, are more in shell fish than in fish, showing the nutritionally superior amino acid make up of fish and shell fish. An important feature of the amino acid composition of prawn is its fairly large content of proline, which is more than twice that in fishes. Proline is considered important in the building of connective tissue such as collagen and elastin which may be more in prawn so as to keep up its body structure with the help of the shells.

Thus there is no major difference between fish and shell fish in its nutrient composition. Prawn is more similar to fish in its proximate and mineral composition, and crab is characterised by higher amounts of moisture, carbohydrate, iron, calcium and less of fat. The only similarity among shell fish being its low fat content and the higher amounts of total essential amino acids, sulphur amino acids and aromatic amino acids, making

them nutritionally better than fish outweighing the higher calorie content of fishes.

The authors are grateful to the late Shri. G. K. Kuriyan, Director, Central Institute of Fisheries Technology, Cochin for encouragement and to Shri P. D. Antony for enlightening discussions.

References

- AOAC (1970) *Official Methods of Analysis* (Horwitz, W., Ed) p. 426. Association of Official Agricultural chemists, Washington
- APHA (1976) *Standard Methods for the Examination of water and Waste water* (Mary Ann Franson, Ed.), 14th edn., p. 208, American Public Health Association, Washington, D. C. 20036
- Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Physio.* **37**, 911
- FAO/WHO (1973) *Energy and Protein Requirements Ser. 522* FAO, Rome
- Fiske, C. H. & Subbarow, Y. (1925) *J. Biol. Chem.* **66**, 375
- Gopakumar, K. (1973) *Studies on Marine Lipids*, Ph. D. Thesis, Univ., Kerala.
- Gopalan, C., Rama Sastry, B. V. & Balasubramanyan, S. C. (1980) in *Nutritive Value of Indian Foods*. p. 94. National Institute of Nutrition, Hyderabad.
- Gowri, V., Vasantha, M. S., Sreenivasan, K. S. & Moorjani, M. N. (1972) *Fish. Technol.* **9**, 180
- Harden, M. L., Stanaland, R., Briley, M. & Yang, S. P. (1976) *J. Fd Sci.* **41**, 1082
- Hawk, B. P. (1954) *Practical Physiological Chemistry*. 13th Edn. p. 545, Mc Graw Hill Book Company, Inc. New York
- James, M. A. (1969) *Sci. Cult.* **35**, 590
- Kavanagh, F. (1963) *Analytical Microbiology*, p. 707, Academic Press, New York
- Kutty Ayyappan, M. P., Shenoy, A. V. & Gopakumar, K. (1976) *Fish. Technol.* **13**, 153
- Mukundan, M. K., James, M. A., Radhakrishnan, A. G. & Antony, P. D. (1979) *Fish Technol.* **16**, 77
- Mukundan, M. K. & James, M. A. (1977) *Fish. Technol.* **15**, 85
- Nair, M. R. & Bose, A. N. (1965) in *Technology of Fish Utilization* (Rudolf Kreuzer, Ed.) p. 68, Fishing News (Books) Ltd. London
- Rangaswamy, J. R., Suryanarayana Rao, S. V. & Lahiry, N. L. (1970) *J. Agric. Fd Chem.* **18**, 298
- Richard, O. B., Elinor, M. R. & Maynard, A. S. (1962) *J. Fd Sci.* **27**, 73
- Smellie, R. M. S. (1974) *Calcium and Cell Regulation*. (Smellie, R. M. S. Ed) p. 29. The Biochemical Society, London
- Sohn, B. I., Joseph, H. C. & George, F. M. (1961) *Comm. Fish. Rev.* **23**, 2
- Thurston & Claude, E. (1958) *Comm. Fish. Rev.* **20**, 1
- Umbriet, W. W., Buriss, R. H. & Stauffer, J. F. (1959) *Manometric Techniques*, p. 239, Burges Publishing Company, Minneapolis-15
- Vogel, I. A. (1960) *A Text Book of Quantitative Inorganic Analysis*, 3rd edn., p. 885, Longman Green and Co. Ltd., London
- White, A., Handler, P. & Smith, E. L. (1973) *Principles of Biochemistry*, 5th edn., p. 459, Mc Graw-Hill Kagakusha Ltd., Tokyo

Selection of Bacterial Flora in the Chlortetracycline Treated Oil Sardine (*Sardinella longiceps*), Indian Mackerel (*Rastrelliger kanagurta*) and Prawn (*Metapenaeus dobsoni*) During Ice Storage*

P. K. SURENDRAN and K. GOPAKUMAR

Central Institute of Fisheries Technology, Cochin-682 029

The native flora of oil sardine and mackerel consisting of *Pseudomonas* spp; *Moraxella* spp; *Acinetobacter* spp. and *Vibrio* spp. underwent significant changes during ice storage. At the time of spoilage, *Pseudomonas* spp. were predominant. CTC treatment significantly reduced the *Pseudomonas* spp. in the initial stages of storage; but later *Pseudomonas* spp. reasserted and constituted the bulk of the spoilage flora. In prawn, the native flora was comprised of *Pseudomonas* spp; *Acinetobacter* spp; *Moraxella* spp. and *Vibrio* spp. At the time of spoilage a heterogeneous flora, consisting of *Pseudomonas* spp; *Moraxella* spp. and *Acinetobacter* spp. predominated. CTC treatment significantly changed the flora of prawns. During spoilage, *Pseudomonas* predominated in CTC treated prawns.

The chlortetracycline (CTC) treatment of fish and prawn enhances their shelf life under refrigerated storage. The CTC affects the growth of sensitive strains of the native flora of fish and prawn, thereby reducing bacterial proliferation and consequent spoilage. Although considerable information is available on the storage characteristics and spoilage of tetracycline treated fish, little effort has been made to examine the resultant microbial population. A knowledge of the effect of antibiotic treatment on the succession of bacterial flora is vital to the preservation of fish by antibiotics. Lee *et al.* (1967) observed no appreciable change in microbial population of CTC treated ocean perch from temperate waters excepting in Gram positive bacteria and yeasts. However, such information from tropical fish and prawn is lacking. The effect of CTC treatment on the bacterial groups at various stages of spoilage of fish and prawn under ice storage are reported in this communication.

Materials and Methods

Fresh oil sardine (*Sardinella longiceps*), mackerel (*Rastrelliger kanagurta*) and prawn

(*Metapenaeus dobsoni*) were procured from fishing crafts operating off Cochin, brought to the laboratory within 2-4 h after they are caught. The fish/prawn were divided into three lots, the first dipped in 5 p.p.m. chlortetracycline (CTC) solution for 10 min, drained well and stored in ordinary crushed ice; the second stored in crushed 5 p.p.m. CTC ice and the third (control) was stored under ordinary crushed ice in thermocole insulated boxes in 1:1 fish to ice ratio. The storage continued for 20-30 days and the meltage of ice was compensated by the addition of respective ice every alternate days. The CTC solution and CTC ice were prepared as described by Surendran (1980). Fish/prawn samples were analysed at the beginning of the study and afterwards at 3-5 days interval.

The total plate count (TPC) was determined using sea water agar (SWA) media. The plates were incubated at $28 \pm 2^\circ\text{C}$ for 3 days. Bacterial cultures were isolated from TPC plates of CTC treated and control samples during each sampling. The isolated cultures after purification by repeated streaking on SWA plates, were maintained on SWA slants for morphological and biochemical characterisation.

*Part of Ph.D. thesis of the first author approved by University of Kerala, 1980.

Morphology and Gram staining (Anon, 1957) were observed in 16–24 h old cultures grown on SWA slants. Presence of spores was detected usually by staining 48–72 h cultures and examining microscopically or by the method of Lee & Pfeifer (1975). Motility was observed by the hanging drop method (Anon, 1957).

The biochemical reactions of the cultures were determined by standard methods (Salle, 1954; Anon, 1957; FDA, 1973), mode of attack of glucose by cultures determined by using the 'ox-ferm' media of Hugh & Leitson (1953), presence of cytochrome oxidase in the cultures by the modified Kovacs' test (1956), catalase by observing under low power of the microscope the evolution of gas when a drop of 3% H_2O_2 (v/v) was mixed with a speck of young culture on a clean slide, pigmentation by observation on sea water-skimmed milk-peptone agar (Surendran, 1980) after a week's incubation at $28 \pm 2^\circ C$, luminescence by examining the cultures in a dark room daily for 4 days after incubation, sensitivity to penicillin (2.5 I.U.) by the pad-plate method and sensitivity of the cultures to CTC determined on peptone beef

extract glucose agar (PBGA) as described by Surendran & Iyer (1971).

The differentiation of the bacterial cultures upto the generic level was done by the scheme (Surendran, 1980) outlined in Fig. 1.

Results and Discussion

Flora of oil sardine

Results of a typical study on the pattern of change in the bacterial flora of oil sardine during ice storage of control and CTC treated samples are given in Table 1.

The native flora of oil sardine underwent significant changes during ice storage. The initial flora consisted of *Pseudomonas* spp. (16%), *Moraxella* spp. (8%), *Acinetobacter* spp. (24%), *Vibrio* spp. (26%), *Flavobacter/Cytophaga* spp. (4%), *Micrococcus* spp. (8%) and others (14%). As the number of days of ice-storage increased, the percentage of *Pseudomonas* spp. increased progressively and after 21 days of ice-storage, 74% of the flora was constituted by *Pseudomonas* spp. alone. During the first few

Table 1. Pattern of change in the bacterial flora of oil sardine (control and CTC treated) during ice storage

	Percentage of microorganisms at different intervals in ice									
	Ordinary ice stored				Dipped in 5 p.p.m. CTC solution and stored in ordinary ice			5 p.p.m. CTC-ice stored		
Days	0	7	14	21	7	14	21	7	14	21
<i>Pseudomonas</i>	16	24	39	74	11	26	80	9	30	87
<i>Moraxella</i>	8	15	11	4	5	7	2	11	6	2
<i>Acinetobacter</i>	24	34	22	8	16	21	7	19	18	5
<i>Vibrio</i>	26	8	5	2	36	20	2	42	24	3
<i>Flavobacter/Cytophaga</i>	4	5	5	2	3	3	1	4	2	0
<i>Micrococcus</i>	8	6	7	4	11	6	3	6	8	1
Others including yeasts	14	8	11	6	18	17	5	9	12	2
Gelatin liquefiers	76	14	26	90	33	30	78	31	20	84
Putrefiers (fish media)	21	24	22	56	11	16	42	7	12	38
Capable of growth at 0°C	18	72	84	95	64	75	97	60	81	98
Sensitivity to 5 p.p.m. CTC	84	76	76	72	51	48	37	47	36	21
No. of cultures identified	82	71	66	72	65	52	57	70	62	67

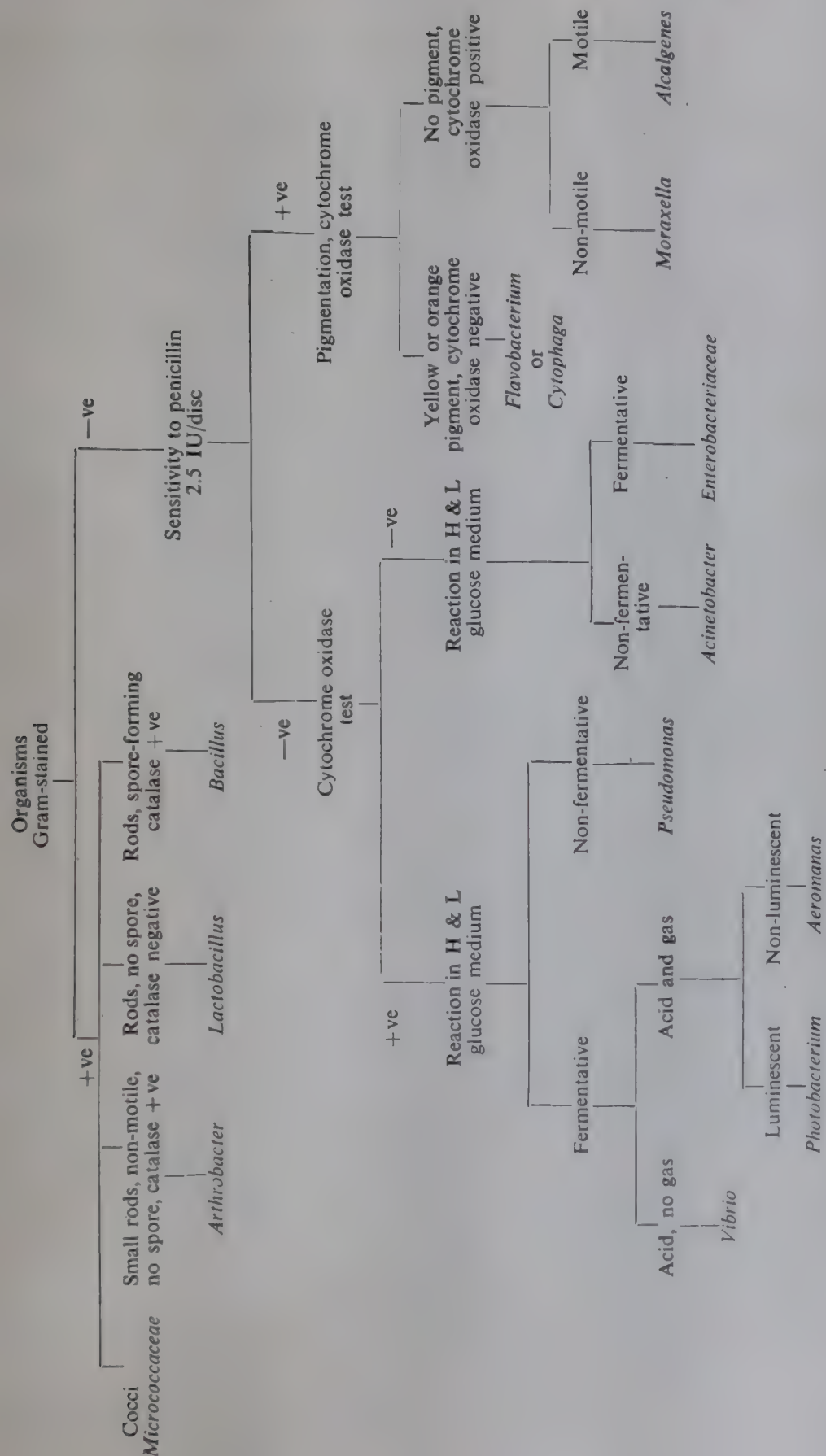


Fig. 1. Scheme used for classifying the cultures

Note:- (1) Gram negative, sensitive to 2.5 I. U. penicillin, no pigment, but cytochrome oxidase negative are *Acinetobacter*-like.
 (2) Penicillin sensitive, Gram negative cytochrome oxidase positive, pigmented yellow or orange, are also grouped as *Cytophaga/Flavobacterium*.

days of ice-storage, there was some increase in the proportion of *Moraxella* and *Acinetobacter* spp. But later, their relative proportion decreased and at the 21st day of ice storage when the fish was spoilt, their share was only 4% and 8% respectively. *Vibrio* spp. which formed 26% of the initial flora, decreased rapidly during ice storage and by the 21st day, their share was only 2% of the total population. Changes in the proportions of *Flavobacter*/ *Cytophaga* and *Micrococcus* were not of much significance.

In fish treated with CTC, either in the form of dips or incorporated in ice, the pattern of change in the flora is quite different. Upto the 7th day of storage, the population is quite heterogeneous in character, the predominant groups being *Vibrios*, yeasts and *Micrococcus*. The *Pseudomonas*, and *Moraxella-Acinetobacter* group were found to decrease. During the next few days, however, the *Pseudomonas* group gradually predominated and by the end of 21 days in ice, more than 80% of the flora of the CTC treated samples were constituted by *Pseudomonas* group. All other groups particularly yeasts and *Vibrios* decreased drastically.

The changes in the flora of control and CTC treated oil sardine, so far as the biochemical groups are considered, were not much different. In the initial flora of fresh fish, 76% were gelatin liquefiers, which in the first few days of ice storage decreased. But later on, the proportion of the flora capable of gelatin hydrolysis increased and by 21st day, 90% of the flora were gelatin liquefiers. The trends in the succession of gelatin liquefiers in the CTC treated samples were more or less similar to that in the untreated control fish.

The percentage of flora capable of putrefaction of fish media, was 21% of the initial flora of fresh oil sardines, which then gradually increased during ice storage to 56% within 21 days in ice. In the case of antibiotic treated samples, there was rapid initial decrease in the proportion of putrefiers. By the 7th day of ice-storage, putrefiers constituted only about 10% in the treated samples, compared to 24% of the control. However, the percentage of

putrefiers gradually increased and by 21 days, 42% of the total flora of the sample dipped in 5 p.p.m. CTC and stored in ordinary ice, and 38% of the flora of the sample kept in 5 p.p.m. CTC, were putrefiers.

The bacteria capable of growth at 0°C, were only 18% of the initial flora of fresh oil sardine, but this fraction increased during storage in ice and by 21 days in ice, 95-98% of the flora of both control and treated fish were capable of growth at 0°C.

The proportion of cultures sensitive to 5 p.p.m. CTC remained more or less in the range of 84-72%, in the case of untreated oil sardine stored in ordinary ice. Whereas, in the case of treated samples, the CTC sensitive flora decreased during storage. This decrease was more pronounced in the case of the fish stored in 5 p.p.m. CTC ice. The flora sensitive of 5 p.p.m. CTC in the case of sardine dipped in 5 p.p.m. CTC solution and subsequently stored in ordinary ice was only 37% and in the case of fish stored in 5 p.p.m. CTC ice was only 21%, by 21 days of storage.

Flora of mackerel

Table 2 presents the typical pattern of change in the bacterial flora of mackerel during ice storage of control and CTC treated samples.

The pattern of the succession of the flora during storage resembled that of oil sardine. Only 16% of the initial flora of fresh mackerel was *Pseudomonas* spp. while 42% was comprised of by *Vibrio* spp. But, as storage in ice proceeded, the proportion of *Pseudomonas* group increased steadily and reached 78% of the total flora within 21 days in ice, whereas *Vibrio* spp. rapidly decreased to 4% by that period. Similarly, major portion (69-80%) of the flora of treated samples on the 21st day of ice storage was constituted by *Pseudomonas* group alone.

The changes in proportion of gelatin liquefiers, psychrophiles, putrefiers and flora sensitive to 5 p.p.m. CTC, were more or less similar to the corresponding changes in oil sardine during ice storage of control and treated samples.

Table 2. Pattern of change in the bacterial flora of mackerel (control and CTC treated) during ice storage

	Percentage of micro-organisms at different intervals in ice									
	Ordinary ice stored				Dipped in 5 p.p.m. CTC solution and stored in ordinary ice			5 p.p.m. CTC-ice stored		
Days	0	7	14	21	7	14	21	7	14	21
<i>Pseudomonas</i>	16	18	32	78	14	31	69	16	27	80
<i>Moraxella</i>	10	16	9	2	9	5	6	14	8	3
<i>Acinetobacter</i>	20	26	14	10	15	11	8	18	21	7
<i>Vibrio</i>	42	22	11	4	36	21	5	32	18	3
<i>Flavobacter/Cytophaga</i>	3	2	4	2	5	4	2	5	4	1
<i>Micrococcus</i>	4	5	8	2	7	11	4	7	8	2
Others including yeasts	5	11	22	2	14	17	6	8	14	4
Gelatin liquefiers	96	16	28	81	22	34	68	18	29	84
Putrefiers (fish media)	19	25	31	46	9	17	32	8	19	28
Capable of growth at 0°C	11	56	65	88	60	68	90	49	63	83
Sensitivity to 5 p.p.m. CTC	90	81	81	69	52	58	46	46	42	28
No. of cultures identified	64	50	60	48	70	40	55	65	61	52

Table 3. Pattern of change in the bacterial flora of prawn (control and CTC treated) during ice storage

	Percentage of micro-organisms at different intervals in ice									
	Ordinary ice stored				Dipped in 5 p.p.m. CTC solution and stored in ordinary ice			5 p.p.m. CTC-ice stored		
Days	0	5	15	25	5	15	25	5	15	25
<i>Pseudomonas</i>	14	16	16	24	9	19	48	6	22	74
<i>Moraxella</i>	20	31	42	40	18	14	12	12	12	6
<i>Acinetobacter</i>	14	22	26	20	11	10	11	14	8	7
<i>Vibrio</i>	18	10	5	2	32	22	9	46	26	6
<i>Flavobacter/Cytophaga</i>	12	6	2	2	6	7	3	5	4	2
<i>Micrococcus</i>	6	4	2	2	8	6	7	5	2	2
Others including yeasts	16	11	7	10	16	22	10	12	25	3
Gelatin liquefiers	80	28	39	85	18	26	69	14	30	91
Putrefiers (fish media)	14	17	37	52	6	15	36	6	22	39
Capable of growth at 0°C	8	46	72	81	24	65	90	30	71	92
Sensitivity to 5 p.p.m. CTC	75	72	81	76	42	47	41	33	19	14
No. of cultures identified	70	65	61	50	56	49	64	48	52	60

Flora of prawn (M. dobsoni)

Pattern of change in the bacterial flora of prawn (*M. dobsoni*) during ice storage of control and CTC-treated samples, is presented in Table 3.

The initial flora of fresh prawn consisted in *Pseudomonas* spp. (14%), *Moraxella* spp. (20%), *Acinetobacter* spp. (14%), *Vibrio* spp.

(18%), *Flavobacter/Cytophaga* spp. (12%), *Micrococcus* spp. (6%) and others including yeasts (16%). The changes in the flora during ice storage was quite different from those of oil sardine or mackerel. While the *Vibrios* decreased rapidly during ice storage to 2% by the 25th day of ice storage, *Pseudomonas*, *Moraxella* and *Acinetobacter* spp. steadily increased and by 25 days in ice, together they constituted 84%

of the flora, the *Pseudomonas* sharing 24%, *Moraxella* 40% and *Acinetobacter* 20%. The results indicated that the spoilage flora of prawn (*M. dobsoni*) stored in ordinary ice, was a mixed group of bacteria, comprising of *Moraxella*, *Pseudomonas* and *Acinetobacter* spp.

But, the patterns of change in the flora of the CTC treated samples were quite different. In the case of prawn dipped in 5 p.p.m. CTC solution, followed by storage in ordinary ice, there was the initial preponderance of *Vibrio* spp., while *Pseudomonas*, *Moraxella* and *Acinetobacter* spp. decreased. Later, *Pseudomonas* spp. began to reassert and by the 25th day of storage they constituted 48% of the flora. Also, *Moraxella* and *Acinetobacter* spp. respectively comprised of 12% and 11% of the flora, the share of *Vibrio* being only 9%.

In the case of prawn stored in 5 p.p.m. CTC ice, the pattern of change of flora was different. In the initial stage of storage in CTC ice, there was a decline in the percentage of *Pseudomonas* spp; accompanied by an increase in the proportion of *Vibrio* spp. Within the first 5 days in CTC ice, *Vibrio* increased to 46% of the flora, while *Pseudomonas* was only 6%; the share of *Acinetobacter* and *Moraxella* spp. being 14% and 12% respectively. By the 25th day in CTC ice, the pattern was considerably different. *Pseudomonas* group formed 74% of the flora, the rest being *Moraxella* spp. (6%), *Acinetobacter* spp. (7%) and *Vibrio* spp. (6%).

It is quite interesting to note that while the normal spoilage flora of prawn (*M. dobsoni*) stored in ordinary ice, consisted predominantly of non pseudomonads namely *Moraxella* spp. (40%) and *Acinetobacter* spp. (20%), *Pseudomonas* spp. constituting only 24%, the spoilage flora of CTC treated prawn were predominantly *Pseudomonas* spp. namely 48% in the CTC dipped and then stored in ordinary ice sample and 74% in CTC iced sample. This meant that CTC treatment brought about a qualitative change in the spoilage flora of prawn, whereas such qualitative changes in the spoilage flora of oil sardine and mackerel were not at all resulted from CTC treatment.

The pattern of change in the proportions of gelatin liquefiers during different intervals of ice storage was similar in the control and treated samples of prawn. However, the incidence of putrefiers in the control and treated samples was different. In the former sample, the putrefiers formed 52% of the flora on the 25th day of ice storage, while the corresponding percentages of putrefiers in the treated samples were 36 to 39%. The incidence of psychrophiles steadily increased during ice storage of control and treated samples. While more than 70% of the flora was sensitive to 5 p.p.m. CTC throughout the entire storage period of the control sample, the proportion of the flora sensitive to 5 p.p.m. CTC steadily decreased in the CTC treated samples. Only 14% of the entire flora of the 5 p.p.m. CTC iced prawn by 25th day, was sensitive to 5 p.p.m. CTC.

Shewan *et al.* (1960) found that the initial flora of North Sea and Faroes cod, mainly consisting of *Pseudomonas*, *Archromobacter*, *Flavobacterium*, *Coryneforms* and *Micrococcus* spp. underwent significant change during storage in ice. *Pseudomonas-Achromobacter* group steadily increased during the initial stages, at the expense of the others and later, by about 15 days in ice, when the fish was in the early stages of spoilage, *Pseudomonas* group emerged as the predominant group, constituting 80-90% of the flora. Similar observations on the succession of genera during ice storage of codling and haddock had been made by Shewan & Stewart (1958), who reported that by 14 days in ice, the *Pseudomonas* spp. constituted 90% of the flora and that most of the active spoilers of fish muscle were members of the *Pseudomonas* group. Shewan (1971) reported that qualitatively, there was little change over the first few days in the bacterial flora of marine fishes such as cod during ice storage, but later, the *Pseudomonas* group particularly III/IV groups gradually took over and by the 12th day constituted 90% of the total flora.

The pattern of changes in the flora of oil sardine and mackerel during ice storage appears to be similar to those of the fishes of temperate waters. In the initial flora, the *Moraxella-Acinetobacter-Vibrio* groups accounted for 50-70% in these fishes, the share of *Pseudomonas* spp. being less than

20%. As the days of ice storage progressed, *Pseudomonas* spp. emerged as the dominant group, accounting for 80-90% of the total flora, at the time of spoilage in ice. This finding is in full agreement with the observation made by Shewan (1977) that irrespective of the initial flora of fish, *Pseudomonas* and *Alteromonas* groups emerged as the predominant genera during spoilage of fish in ice-storage. This has been attributed to the shorter generation times of these *Pseudomonas* and *Alteromonas* spp. at chill temperatures (Harrison-Church, quoted by Shewan, 1977) compared to other groups of bacteria.

In the case of codling from North Sea, stored in 5 p.p.m. CTC ice, the bacterial population upto the 8th day was quite heterogeneous in character, the predominant groups being *Vibrios* and yeasts, *Pseudomonas* and *Achromobacter* groups constituting less than 25% of the total flora. However, the *Pseudomonas* group gradually predominated in the next few days and by the 16th day, the normal type of spoilage flora comprising of 79% of *Pseudomonas* spp. was established (Shewan & Stewart, 1958; Shewan, 1962 a). The findings reported in this paper regarding the changes in the flora of oil sardine and mackerel during storage in 5 p.p.m. CTC ice, are in agreement with the above findings. While 24% of the flora of the untreated sardine on the 7th day of ice storage was *Pseudomonas* spp. only 90% of the flora of the sample kept in 5 p.p.m. CTC ice was *Pseudomonas* spp. (Table 1). CTC treatment had brought about a significant reduction in the number of *Pseudomonas* spp. which comprised most of the active spoilers of fish. But, of course, the *Pseudomonas* spp. reasserted later and after a lapse of 7-10 days emerged as the dominant group constituting about 80-90% of the total flora. Hence, as observed by Shewan (1961, 1962 a) and Shewan & Stewart (1958), the extension of shelf life of 7-10 days obtained by CTC treatment, appeared to be the time required for the normal flora to recover from the effects of the antibiotic. But this advantage of the effect of CTC on bacterial flora, was of no avail for the preservation of oil sardine and mackerel, since their high fat content contributed to chemical

spoilage of fat leading to rancidity of the muscle.

The changes in the bacterial flora of Gulf of Mexico shrimp during storage in crushed ice had been reported by Campbell & Williams (1952), who showed that irrespective of the composition of the initial flora, being heterogeneous in character, the flora of the shrimp after 16 days in ice, was composed of 82% of *Achromobacter* spp. and 16.5% of *Pseudomonas* spp. Walker *et al.* (1970) have found that even though 80% of the initial flora of the Dublin Bay prawn-Scampi (*Nephrops norvegicus*), was constituted by 'coryneforms' during ice storage the flora changed and finally consisted of 70% *Achromobacter* spp. and 8% *Pseudomonas* spp. In the case of tropical prawns from Mosambique, the spoilage flora after 12 days in ice, consisted of 67% *Achromobacter* spp., 19% *Coryneforms* and 8% *Flavobacterium/Cytophaga*, while the flora of prawns from Malayasia after 12 days in ice, comprised of 48% *Achromobacter* spp. and 41% *Coryneforms*, the *Pseudomonas* forming only 8% (Cann, 1974).

The authors' finding that 60% of the flora of Indian prawn (*M. dobsoni*) after 25 days in ordinary ice, was constituted by *Moraxella-Acinetobacter* group (the erstwhile *Achromobacter* group), very well agrees with the findings of Campbell & Williams (1952), Walker *et al.* (1970) and Cann (1974). But, it differs from the spoilage flora of Gulf of Thailand prawn (*Peneaus* spp.) where 90% of the spoilage flora was composed of *Pseudomonas* spp. and only 4% by *Achromobacter* spp. However, 24% of the flora of Indian prawns (*M. dobsoni*) (Table 3) at the time of spoilage in ice was comprised of *Pseudomonas* spp. also.

The observation that the spoilage flora of prawn in ice storage, comprised of 60% *Moraxella-Acinetobacter* group and only 24% *Pseudomonas* spp. clearly shows the heterogeneous nature of the spoilage of flora of prawn. In this respect, it is significantly different from the spoilage flora of fishes, both tropical (Tables 1 and 2) and temperate water fishes, in which cases, the spoilage of ice stored fishes, is brought about by predominantly *Pseudomonas* spp., the contribution by other groups, if at all, being insignificant.

The finding that the spoilage flora in the CTC iced prawn was, mainly comprised of *Pseudomonas* spp. is very significant. It evidently shows that there is a very important qualitative difference in the spoilage flora between the prawn stored in ordinary ice and antibiotic ice. As evident from earlier discussions, *Pseudomonas* spp. constituted the major spoilage flora of fishes. Hence, the emergence of *Pseudomonas* as the major spoilage flora of CTC stored prawn indicated that, there might be some difference in the spoilage of antibiotic treated and untreated prawn. The end products of spoilage in both cases may be different, both qualitatively and quantitatively.

CTC sensitivity of cultures from CTC treated fish and prawn

The CTC sensitivities of the bacterial types present on fish and prawn underwent appreciable changes during the storage of the fish and prawn in the antibiotic ice. Typical results on the CTC sensitivities of bacterial cultures isolated from CTC ice stored oil sardine are presented in Table 4.

In the case of the cultures isolated from oil sardine just before CTC storage, majority of the cultures belonging to *Pseudomonas*, *Moraxella*, *Acinetobacter*, *Micrococcus*,

Flavobacter/Cytophaga and *Alcaligenes* spp. were sensitive to 5 p.p.m. CTC and 100% of these cultures were sensitive to 25 p.p.m. CTC. But, only 32% each of *Vibrio* and *Archrobacter* strains were sensitive to 5 p.p.m. CTC.

When the number of days of storage in CTC-ice increased, there was evidently an increase in the percentage of insensitive flora. After 7 days in CTC-ice only 47% of *Pseudomonas* spp., 65% of *Moraxella* spp., 50% of *Acinetobacter* spp. and 49% of *Micrococcus* spp. were sensitive to 5 p.p.m. CTC.

Sensitivity to CTC further decreased in the case of cultures isolated from fish stored in CTC ice for 21 days. Only 21% of *Pseudomonas* spp., 32% of *Moraxella* spp., 26% of *Acinetobacter* spp. and 39% of *Micrococcus* spp. were then sensitive to 5 p.p.m. CTC.

Development of insensitive flora during the course of CTC storage of fish is very significant so far as the prospect of CTC being used as a preservative is concerned. Shewan & Stewart (1958) and Shewan (1962 b) also observed that, while only 1% of the original flora of codling and haddock, was insensitive to 5 p.p.m. CTC, by the 16th day of storage in CTC ice, about 90% of the total

Table 4. CTC sensitivity of bacterial cultures isolated from CTC ice stored oil sardine

Bacterial genus	Percentage of cultures sensitive to the given CTC levels											
	Cultures from sardine before treatment				Cultures from sardine 7 days in 5 p.p.m. CTC ice				Cultures from sardine 21 days in 5 p.p.m. CTC ice			
	No. of cultures	CTC p.p.m.			No. of cultures	CTC p.p.m.			No. of cultures	CTC p.p.m.		
		1	5	25		1	5	25		1	5	25
<i>Pseudomonas</i>	60	61	76	100	64	24	47	100	52	4	21	68
<i>Moraxella</i>	71	54	82	100	65	51	65	100	62	16	32	74
<i>Acinetobacter</i>	48	55	78	100	50	20	50	100	54	10	26	70
<i>Vibrio</i>	80	16	32	80	66	12	25	60	50	4	16	44
<i>Micrococcus</i>	24	50	96	100	30	33	49	100	32	18	39	82
<i>Flavobacter/</i>												
<i>Cytophaga</i>	16	42	72	100	20	25	75	100	20	10	60	100
<i>Alcaligenes</i>	10	40	100	100	16	24	72	100	12	16	48	80
<i>Arthrobacter</i>	12	8	32	64	16	6	24	66	10	9	20	50

flora and 100% of *Pseudomonas* spp. were insensitive. Lee & Sinnhuber (1967) have found that the proportion of CTC resistant species increased with higher CTC concentration and the length of storage at 7°C. They found that among individual generic groups isolated from CTC treated ocean perch, 'coryneforms' and yeasts were more resistant to CTC and that *Pseudomonas*, *Achromobacter*, *Flavobacterium*, *Bacillus* and *Lactobacillus* all contained species either resistant or sensitive to CTC. More CTC resistant species in these genera accumulated with the increased CTC concentrations and with the length of storage in presence of CTC. The authors' findings that *Arthrobacter* spp. (formerly grouped as 'coryneforms') constituted the most insensitive group and that, the CTC resistance of other groups of bacteria increased with the length of storage in presence of CTC, are in full agreement with the observations of Lee & Sinnhuber (1967).

The authors are thankful to the Director, Central Institute of Fisheries Technology, Cochin-682029 for the facilities provided for the work.

References

- Anon (1957) *Manual of Microbiological Methods*. Society of American Bacteriologists. McGraw Hill Book Co. Inc. New York
- Campbell, L. L. (Jr) & Williams, O. B. (1952) *Fd Technol.* **6**, 125
- Cann, D. C. (1974) *Bacteriological Aspects of Tropical Shrimp*. in *Fishery Products* (Kreuzer, R., Ed.), p. 338, Fishing News Books, Surrey.
- F. D. A. (1973) *Bacteriological Analytical Manual for Foods* - Chapter XIX. *Examinations of Shell Fish and Meats*. Division of Microbiology, Bureau of Foods; Food and Drug Administration, U. S. A.
- Hugh, R. & Leifson, E. (1953). *J. Bact.* **66**, 24
- Kovacs, N. (1956) *Nature*, **178**, 703
- Lee, J. S. & Pfeifer, D. K. (1975) *Appl. Microbiol.* **30**, 72
- Lee, J. S. & Sinnhuber, R. O. (1967). *Appl. Microbiol.* **15**, 543
- Lee, J. S., Willet, C. L., Robinson, S. M. & Sinnhuber, R. O. (1967) *Appl. Microbiol.* **15**, 368
- Salle, A. J. (1954) *Laboratory Manual on Fundamental Principles of Bacteriology*. 4th edn. McGraw Hill Book Co. Inc. New York
- Shewan, J. M. (1961). *The Microbiology of Seawater Fish*. in *Fish as Food*. (Borgstrom, G., Ed.) Vol. 1, P 487, Academic Press, New York
- Shewan, J. M. (1962 a) *The Bacteriology of Fresh and Spoiling Fish and Some Related Chemical Changes*. in *Recent Advances in Food Science* (Hawthron & Muil Leitch eds.) p. 167, Butterworth, London
- Shewan, J. M. (1962 b) *The Use of Antibiotics in the Preservation of Fish*. in *Antibiotics in Agriculture*. (Woodbine M., Ed) p. 289, Butterworths, London.
- Shewan, J. M. (1971) *J. Appl. Bact.* **34**, 299
- Shewan, J. M. (1977) *The Bacteriology of Fresh and Spoiling Fish and the Biochemical Changes Induced by Bacterial Action* in "Proc. Trop. Prod. Inst. Conf. Handl. Process. Mark. Trop. Fish." 1977, 511, London, July 1976.
- Shewan, J. M. & Stewart, J. (1958). *Experiments in the Use of Antibiotics in fish Preservation*. Proc. Intern. Inst. Refrig. (Moscow). DSIR Food Investigation Memoir no. 1203. 15 pp.
- Shewan, J. M., Hobbs, G. & Hodgkiss, W. (1960) *J. Appl. Bact.* **23**, 463
- Surendran, P. K. (1980) *Chemical Preservatives in Relation to Control of Microbial Changes in Fishery Products*. Ph.D. Thesis, University of Kerala, Trivandrum
- Surendran, P. K. & Iyer, K. M. (1971) *Fish. Technol.* **8**, 128
- Walker, P., Cann, D. & Shewan, J. M. (1970) *J. Fd Technol.* **5**, 375

NOTES

On the Vertical Distribution of Seers and other Commercially Important Fishes in the Surface Drift Nets

Amongst the various factors considered while designing a suitable gill net the swimming height of the fishes to be caught assumes paramount importance. Studies on this aspect have been made by Parrish (1963) Berst & Mcombie (1963), and Sulochanan & Rao (1964) for perches and pomfrets. This communication highlights the authors' attempts to determine the vertical height for catching seers and other commercially important fishes off Kakinada coast.

For a detailed description of the net attention is drawn to the paper on mesh selectivity studies for spotted seer (Sreekrishna *et al.* 1972). The fishing height of the net was kept constant at 6 m. To determine the swimming height of the fishes, the nets were divided horizontally into six sections by passing coloured twines at every 1 m interval. Twenty shots of nets were operated on all fishing days.

The number of *Scomberomorus commerson*, *S. guttatus*, *Parastomatous niger*, *Euthynnus affinis*, *Hilsa toli* and Sharks

recorded from each 1 m division of the net together with statistical significance are presented in Table 1. Out of 247 *S. guttatus* and 87 *S. commerson* caught, majority were found to be in 0-3 m depth of the net with maximum at 1-3 m. The chi-square (X^2) test indicated (Table 1) that the number of fishes caught in the different sections of the net were significantly different. It was 1% level for *S. commerson* and *H. toli* and 0.1% in *S. guttatus*, *P. niger* and sharks. For *S. guttatus* and sharks 1-3 m, for *S. commerson* 1-2 m, for *P. niger*, *H. toli* and tuna, 0-3 m gave significantly higher catch over the other sections. Berst & Mcombie (1968) observed abundance of perches and sucker fish towards the foot rope of the net while Sulochanan & Rao (1964) emphasised the superiority of the first quarter of the net of 467 cm depth in catching silver pomfrets.

The results of the present studies confirm the view that of the 6 m depth of the net, 0 to 3 m is most efficient, 3 to 5m also contribute substantially to the total catch, while 5 to 6 m is least effective for catching different species of fish investigated.

Table 1. Vertical distribution of fishes in experimental gill nets

Species	Distance from head rope (m)						Total	Observed chi-square value	D.F.	Level of signifi- cance
	0-1	1-2	2-3	3-4	4-5	5-6				
<i>S. guttatus</i>	42	73	74	33	18	7	247	93.83	6	0.1%
<i>S. commerson</i>	9	39	19	10	10	—	87	18.34	4	1%
<i>P. niger</i>	134	199	135	56	49	8	581	259.49	5	0.1%
<i>H. toli</i>	10	22	12	6	1	1	52	11.78	3	1%
<i>E. affinis</i>	57	93	69	29	22	6	276	115.73	5	0.1%
Shark	52	100	108	52	45	11	368	108.38	5	0.1%

The authors are thankful to the late Shri G. K. Kuriyan and Shri K. A. Sadanandan for reading the manuscript and offering suggestions.

References

Berst, A. H. & Mcombie, A. M. (1963)
J. Fish. Res. Bd Can. **20**, 755

Parrish, B. B. (1963) *Int. Commn. North-Atlantic Fish. Spl. Pub.* **5**, 165

Sulochanan, P. & Rao, K. K. (1964)
Indian J. Fish. **9B**, 9

Sreekrishna, Y., Rao, J. S., Percy Dawson,
Mathai, T. J. & Sulochanan, P. (1972)
Fish. Technol. **9**, 133

Kakinada Research Centre of
Central Institute of Fisheries
Technology, Kakinada

PERCY DAWSON
J. SITARAMA RAO

&

Y. SREEKRISHNA

BOOK REVIEW

Study of the Sea

The Development of Marine Research Under the Auspices of the International Council for the Exploration of the Sea

Edited by

E. M. THOMASSON

Fishing News Books Ltd., England

Price: £ 25.00, 272 Pages & 72 illustrations

'Study of the Sea' is a fascinating book, designed primarily for the non-specialist, containing a collection of 40 well chosen papers by leading marine scientists. This interesting collection, conceived and compiled by Mr. E. M. Thomasson, Librarian of the International Council for the Exploration of the Sea (ICES) in Copenhagen, provides a unique survey of some of the most important activities in marine sciences pursued under the council's umbrella. As has been aptly stated by Dr. B. B. Parrish, the President of the Council in his forward to the volume "It traces through a careful and well balanced selection of papers from the Council's archives, the history of major development and exciting events in these fields since the Council was founded at the beginning of the century."

These essays highlight in an entertaining and illuminating way many of the leading problems and developments in oceanographic and fishery sciences during the past 80 years. The editor's explanatory notes prefacing each of the paper together with the ideas expressed by these famous early pioneers help to show clearly the magnitude and complexity of some of the problems and the perspective thinking of these marine scientists in setting the stage, and assessing the scope for international co-operation in their investigations towards the understanding of the seas and their living resources.

The ICES was founded in 1902 from a desire to foster international co-operation

in marine biology and hydrography and to conserve North Sea fish stocks. It has been active in the field of oceanography and is the oldest intergovernmental marine organisation in the world.

From a humble beginning with a few scientists representing eight north European countries, it has grown over the years as an effective and large co-ordinating and advisory body involving hundreds of scientists from 18 countries on both sides of the Atlantic.

The papers are arranged mostly in chronological order. Starting with the formative years of the European marine science and the aims of the ICES, the essays include among others classical contributions of such well known persons as Fridtjof Nansen, Johan Hjort, Otto Pettersen, M. Knudsen, Johannes Schmidt, Friedrich Heincke, Harald Sverdrup and Sir Alister Hardy. These were thoughtfully picked out from the Council's archives and an effort has been made by the compiler to give special emphasis on those problems that are of concern such as overfishing, migration, transplantation, pollution and fluctuations in fisheries. Carefully avoiding historical, controversial and highly technical articles, the editor quite rightly preferred to provide a "panoramic picture of what scientific research is like carried out under the auspices of an international organisation that concerns itself with one of mankind's most important resources, by presenting the

work of those who have participated in it over the years."

Fascinating accounts are presented, among others concerning the early experiences with the echo-sounder, and the adventurous saga of Nansen's career, vividly describing the 'vast and expensive enterprise' of scientific research at sea and the "unique and rare combination of personal qualities in those who undertake this work."

Besides the thought provoking essays, the book has also reproduced two historic photographs of great interest namely the group photos of the participants of the 1904 and 1924 ICES meetings which include several world famous marine scientists.

This book will undoubtedly be liked and enjoyed by all who have an interest in the marine sciences and its history and should find a place in all libraries.

PROF. N. BALAKRISHNAN NAIR

SYMPOSIUM ON HARVEST AND POST HARVEST TECHNOLOGY OF FISH

24-27 NOVEMBER, 1982

The Society of Fisheries Technologists (India) is convening a symposium on 'Harvest and Post Harvest Technology of Fish' during 24-27 November, 1982 at Cochin. The symposium will be held under the following technical sessions.

1. Fishery resources
2. Fishing crafts
3. Fishing gear and methods
4. Machinery, equipment and instrumentation
5. Handling and transportation of fish
6. Biochemistry and bacteriology of fish preservation and storage
7. Fish processing
8. Fishery products and byproducts
9. Fish inspection and quality control
10. Extension and education in fisheries

Experience papers contributed to the symposium should present unpublished data and should be prepared under any one or more of the above topics. Manuscripts prepared in the format (a) Abstract (b) Introduction (c) Materials and Methods (d) Results and Discussion (e) Acknowledgement, if any and (f) References may ordinarily be limited to 15 typed pages in case of experience papers and 25 pages in case of review papers. An abstract in not more than 200 words depicting salient features and results of investigations should be sent to reach the Convener not later than May 31, 1982. The full text may reach the Convener by 31st August, 1982.

A registration fee of Rs. 25/- will be charged per participant from India and U.S. \$ 25.00 or its equivalent per participant from abroad. Registration fee for bona-fide members of the Society will be Rs. 15/-. Cost of travel, accommodation, boarding, tourist excursion etc. will have to be borne by participants or their sponsoring organizations.

